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THE JOURNAL OF HYGIENE

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THE JOURNAL OF HYGIENE

INTRODUCTION

THE *Journal of Hygiene* has been founded in order to meet a need which has long been felt for a journal devoted to the publication in the English language of original work in Hygiene. The results of such work in Great Britain, the Colonies, and the United States, have hitherto for the most part appeared in journals devoted to General Medicine, Pathology, Physiology, and Chemistry, or in official Reports and other publications which are not readily accessible for reference. The papers in question have, in consequence, frequently required to be unduly curtailed in the description and discussion of essential data, and have often escaped the notice of workers in the same subject, both in English-speaking and foreign countries. The numerous encouraging assurances of support received from those gentlemen who have kindly consented to collaborate with us have confirmed us in the opinion that the *Journal of Hygiene* will fulfil a definite purpose by serving as a focus to English-speaking investigators for work in Physics, Chemistry, Physiology, Pathology, Bacteriology, Parasitology, and Epidemiology, in relation to Hygiene and Preventive Medicine.

With a view to increasing the general usefulness of the *Journal of Hygiene* we propose not to limit the contributions entirely to reports of original observations and experiments, but to accept and encourage discussions of administrative and practical questions, the importance of which is apt to be overlooked in scientific journals. We also contemplate the occasional publication of collective and critical reviews upon subjects of general interest in the domain of Hygiene, these papers to be accompanied by adequate bibliographical references. Although the list of collaborators is limited to those whose native tongue is English we trust that foreign investigators will understand that their contributions will be welcome.

We conclude this brief introduction with a letter expressing his approval and good wishes from a veteran leader in the Science of Hygiene, Sir John Simon.

GEORGE H. F. NUTTALL

JOHN HALDANE

ARTHUR NEWSHOLME.

40 KENSINGTON SQUARE, W.

Monday, 22nd October, 1900

GENTLEMEN,

My eminent friend Sir John Burdon Sanderson has given me the very interesting information that you purpose to inaugurate the beginning of the new century by founding a scientific *Journal of Hygiene*; and in giving me this information he conveys to me your very flattering request that I should be a contributor to its first number. In the partiality of his old friendship for me, and perhaps with some touch of that poetical imagination which is said to be half-sister of science, my valued friend has forgotten that I am now 84 years of age; and to this I have to add the equally serious disqualification that during the last few months increasing blindness has rendered me (as you see) incapable of guiding my pen for more than the signature of my name. Apart therefore from other insufficiencies of which I am conscious, I feel obliged by age and physical incapacity to decline undertaking what, had I the requisite ability, I should esteem it a signal honour to attempt.

May I venture a word of congratulation on the work you are about to commence, and on the moment you have chosen for commencing it? The past two centuries, and especially the last fifty years, have been beyond measure progressive in the departments of knowledge to which your undertaking relates; mares-nests in science are tending to be a constantly diminishing quantity; and the time seems now to have come when a new Journal edited by representative men of Oxford, Cambridge, and London, may surely expect to escape pitfalls, and to represent in the main what shall prove to be advances in true knowledge.

I beg to remain

Gentlemen

Most faithfully yours

JOHN SIMON.

To Dr G. H. F. NUTTALL

Dr JOHN HALDANE, F.R.S. and

Dr ARTHUR NEWSHOLME.

STUDIES IN RELATION TO MALARIA.

I.

THE GEOGRAPHICAL DISTRIBUTION OF ANOPHELES IN RELATION TO THE FORMER DISTRIBUTION OF AGUE IN ENGLAND.

(Two Maps.)

By GEORGE H. F. NUTTALL, M.A., M.D., PH.D.,
University Lecturer in Bacteriology and Preventive Medicine, Cambridge;

LOUIS COBBETT, M.A., M.D., F.R.C.S.

AND

T. STRANGEWAYS-PIGG, M.A., M.R.C.S.
Demonstrator in Pathology, Cambridge.

(From the Pathological Laboratory of the University of Cambridge.)

GRASSI has repeatedly asserted that the geographical distribution of the genus *Anopheles* in Italy coincides with that of malaria. Even in his most recent publication he lays special stress upon the coincidence, and considers that what he claims will probably hold true all over the world.

Grassi (1900, p. 35) writes, "Confrontando i risultati, ottenuti nelle più differenti parti d'Italia, ho potuto facilmente rilevare che *nei luoghi malarici vi sono dei mosquitos particolari che mancano nei luoghi non malarici. La loro quantità è in complesso in proporzione diretta col numero dei casi di malaria.*"

"Non trovai, per quanto io abbia accuratamente cercato, alcun luogo di pianura in Italia dove prosperino i mosquitos propri de' luoghi malarici e non si dia malaria." Another passage (p. 50) reads, "*Nei luoghi malarici vi sono veramente degli animali speciali succhiatori di sangue che non si trovano nei luoghi malarici*." Still another passage reads (p. 51), "Tutto ciò che riferii per le regioni malariche d'Italia vale probabilmente per tutte le plaghe malariche del mondo²."

¹ The italics are Grassi's.

² See Bibliography at the end of the following paper by Nuttall and Shipley (p. 75).

Basing his deductions upon this statement which he makes, as he says after careful investigation, he proceeds on the strength of their wider geographical distribution to exclude a number of blood-sucking animals: *Gnathobdellidae*, *Ixodinae*, *Argasinae*, *Muscinæ*, *Tabanidae*, *Simuliidae*, *Phlebotominae*, *Ceratopogonidae*, *Pulicidae*, *Pediculidae*, *Acanthiadae* and *Culicidae* (genus *Culex*) from being possible carriers of the parasites of malaria. He states that there are 23 species of *Culicidae* known in Italy, 19 of which belong to the genus *Culex*, and 4 to that of *Anopheles* (*A. pseudopictus*, Grassi 1899, *A. superpictus*, Grassi 1899, *A. claviger*, Fabr. 1805 vel *maculipennis*, and *A. bifurcatus*, L. 1758, the last-named species being identical according to Ficalbi and Grassi with *A. villosus* 1827, *A. plumbeus* 1828, and *A. nigripes* 1839)¹.

The statement of Grassi seemed to gain support from the observations of Macdonald (16 Sept. 1899) in southern Spain. He searched for *Anopheles* in 12 districts, 9 of which were malarious. In the 3 healthy districts he only found *Culex*, in all of the others he detected *Anopheles*. (*A. maculipennis* in all cases, *A. pictus* in three, and *A. bifurcatus* once.)

We do not doubt but that the number of *Anopheles* present in a district will be found to agree with the extent to which malaria prevails, but the investigations which we are about to report show very clearly that Grassi's claim has no general application, for we have found *Anopheles* in many parts of England where there is no record of malaria having previously existed, and where there is certainly no malaria to-day².

General observations upon the geographical distribution of Anopheles, and their mode of dissemination.

Members of the genus *Anopheles* are being found all over the world, and a number of observers are reporting their presence in malarious districts in various countries. In a monograph which will shortly appear from the pen of Mr F. V. Theobald some 42 species or more will be described. Confining ourselves to the three species which are known to occur in England we find that *Anopheles maculipennis* is by far the most prevalent species in this country and in other parts of Europe, and apparently in America. This species has been found in England, Scotland, Wales and Ireland, as will be seen by reference to the following

¹ See footnote ² on p. 47.

² Note whilst going through the press: Celli (*Centralbl. f. Bakteriologie*, Vol. xxviii. p. 534, 5 Nov.) reports observations in Italy which confirm ours. He found *Anopheles* in healthy and elevated situations where there has never been malaria.

tables. It has been found in Scandinavia (Zetterstedt), Germany (Meigen, Koch, and one of us), Austria (Schiner), Russia (Gimmerthal), Holland (van der Scheer), Denmark (Meinert), in many parts of Italy and the adjacent Islands (Ficalbi, Grassi, etc.), as also in the United States (Howard, etc.) and Canada. *Anopheles bifurcatus*, which is less numerous, has been found in Great Britain, Lapland (Zetterstedt), Russia and Italy (Ficalbi), though it will probably be found elsewhere when searched for. *Anopheles nigripes*, which is the least frequent of these species, is found in Great Britain and Italy, and will doubtless be also found elsewhere.

In referring to *A. maculipennis* Grassi (1900, p. 58) states that it may be considered almost a domestic species because of the fact that the imago is frequently found in houses, sheds, stables, and chicken-coops in Italy. This is especially the case in the cooler season, when the flies which seem to prefer the warmth of the house congregate there with a view to hibernating. When the weather grows cool in autumn the flies are also not infrequently found beneath bridges in northern Italy. In a letter to one of us (31 May, 1900) Theobald states that most of this species have been taken by him in England in outdoor closets and sheds, especially in spring, when the insects come from their winter quarters. We have on several occasions found the fly in houses, and thrice in an outdoor closet and cellar respectively in Cambridge during July, October and December. As the result of his experience in collecting *Anopheles* in Scotland and Herefordshire this last summer Lieut.-Colonel Yerbury, F.E.S. writes to one of us (Oct.) that "The natural home of *Anopheles* seems to be damp, swampy ground but not necessarily so wet as marsh or fen." According to Grassi and Ficalbi *A. maculipennis* occurs most frequently in flat land in Italy, the larvae generally requiring clear water rich in vegetable food. The imago feeds upon plant juices, as also upon the blood of man and the domesticated animals. Grassi (1900, p. 93) states that it is hard to catch *A. bifurcatus* and *A. superpictus* except when they are in the act of sucking blood. *A. pseudopictus*, which is found in Italy but not in the adjacent islands, is rarer than *A. bifurcatus*, and prefers the open country, especially land covered with rushes. This species is not encountered in houses and is difficult to catch except when biting. *A. superpictus* is found in houses etc. like *A. claviger*.

Other species of *Anopheles* have been observed to frequent dwellings. Christophers and Stephens (Aug. 1900, p. 6) found an undescribed species of *Anopheles* in huts in Sierra Leone, its numbers gradually

diminishing with the drying up of the adjacent pools, this dropping off in numbers being especially noticeable two weeks after the pools had dried. The flies exhibited a preference for dark, native huts. Stephens and Christophers (July, 1900, p. 56) found that these insects were apparently attracted by the odour emitted by the natives, for it was observed that when natives slept in a tent previously used by Europeans the insects congregated there. In a tent occupied by Europeans usually 2 *Anopheles* were found on inspection in the morning. On the first morning after this tent was occupied by natives 19 insects were captured, and on the second morning no less than 62. The number of *Anopheles* rapidly fell after the tent was disused by natives. Returning to Italy we find Grassi (31 Aug. 1899) reporting the following with regard to the influence of elevation upon the local distribution of *Anopheles*. He found fewer *Anopheles* in the upper stories of a house situated in a malarious district. (This summer one of us made a similar observation in a country house on the Rhine, the same being situated in a district where there had been no malaria for 23 years.) Grassi found *Anopheles* to be frequent in low-lying huts, whereas they were absent in neighbouring huts situated at an elevation but 2—3 meters higher than the others. He states that this distribution is less evident where the insects are numerous and hungry. The observation that persons living in the upper stories of a house are relatively exempt from malaria is an old one. Grassi (17 Sept. 1899) found *Anopheles* in houses at Sermoneta and Sezze, especially those situated in low ground and facing the Pontine Marshes. Sermoneta lies at an elevation of 257 meters and is supplied with *Anopheles* from pools below situated at an elevation of 16 meters. The *Anopheles* (imago) at Norma (343 meters) were bred at Ninfa (24 meters). Similarly at Sezze (319 meters) the flies were bred in marshy pools at Le Fontane (230 meters) and in other places below the town. Christophers and Stephens (Aug. 1900, p. 6) believe that the *Anopheles* observed in Sierra Leone may fly to a distance of a quarter of a mile or more. In Freetown they however found the flies scarce in dwellings situated at a distance of 100—200 yards from breeding-pools. They believe that in certain cases (p. 10) the insects must have flown a distance of 300—600 yards. During the dry season Stephens and Christophers (p. 48) found very few flies in houses, and could only determine their presence in Freetown by constructing artificial test pools in which eggs were promptly laid. In the bush along the Sierra Leone Railway (p. 51) the flies were found to congregate in native huts and villages, though

there were no breeding-places distant less than a quarter of a mile. *Anopheles* were also found in the bush (p. 53) during the dry season, breeding-places being situated at considerable distances.

In Nuttall's monograph (1899) reference is made to the probable influence of winds, railways and ships in the dissemination of mosquitoes. It is stated therein that Roe once observed about a dozen foreign species of mosquitoes on board a ship lying at quarantine in New York. The occurrence of *Anopheles* in railways has since been observed by Grassi in Italy, and Howard (1900, p. 15) dwells upon this mode of dissemination, stating that he knows of one instance in the Catskill Mountains in New York "where the infestation of a previously uninfested place could have been brought about in no other way." It is certain that trains passing through mosquito-infested districts will aid in the dissemination of these insects. Grassi (1900, p. 223) moreover relates how he caught some 200 *Anopheles* on the inside of a coach during a drive lasting two hours through the plains of Capaccio, many *Anopheles* resting within the vehicle and being thus transported. Referring to *Culicidae* in general, Howard (p. 13) cites Smith as stating that the flies would not rise and take flight when a stiff breeze is blowing, and that even a comparatively slight breeze will keep them from the upper stories of a house. On the other hand he writes that Fernald (at a meeting of the Am. Soc. of Economic Entomologists) describes an observation to the contrary. Fernald saw no mosquitoes at Cold Spring Harbour, Long Island, whilst a north breeze blew, whilst they appeared with a gentle south wind after it had blown five or more hours, which led him to conclude that the mosquitoes had been blown 15 miles from the south shore. That some species of *Culex* are limited in their power to disseminate themselves solely by their flight is indicated by an observation of Reese in Baltimore, who found that the number of mosquitoes in his house was greatly reduced after he had treated his privy vault with kerosene, breeding-places being situated close by. Moreover Osborn noted at Ames Harbour that mosquitoes disappeared from the College buildings when small pools within a radius of one quarter to half a mile became dried. In the latter case there were pools at about a distance of a mile which did not cease to be breeding-places. It is evident from this brief summary of what has been observed that it will be necessary to make more exact studies of the methods of dissemination of these insects. What applies to one species may not apply to another. There is one mode of distribution which one of us thinks of considerable importance,

though it does not seem to have struck others. We refer to the dissemination along the course of rivers down which eggs, larvae, and pupae, may be carried, as we shall see from the following tables. If present at the head waters of a stream the insects certainly are carried even down to the estuary, coming to maturity all along the course of the river wherever there is a backwater, a recess in the bank containing still water and frequently accumulation of weed, or wherever the river water overflows into neighbouring ditches.

It seems in place here to mention the little that is noted in the literature with regard to the numbers of *Anopheles* observed to be present in various localities. Here again we shall see that rough numerical estimates would be of value. We have noted above that Grassi caught no less than 200 *Anopheles* inside a coach during a drive lasting two hours, the road leading through a malarious region in Italy. An editorial in the *British Medical Journal* (27 Oct., 1900, p. 1266) states that Sambon counted as many as 20 *Anopheles* larvae in 1 c.c. of water taken from a pool on the Roman Campagna. Though Celli and Delpino (Oct. 1899, p. 8) give no numbers, they state that the number of fresh cases of malaria in Italy coincides with an increase in the number of *Anopheles* (flies) found in and about the houses. They found larvae in March, blood-filled insects in July and August, during which time the number of flies increased. The first infected flies were caught in June. Stephens and Christophers (July 1900, p. 43) working at Freetown, Sierra Leone, found that coincident with the decrease of malaria "there occurs a diminution in the numbers both of the breeding-grounds and the adult insects of *Anopheles*." They draw especial attention to the fact (p. 47) that *Anopheles* are frequently present in enormous numbers in overcrowded native dwellings in Freetown. Christophers and Stephens (Aug. 1900, p. 9) found that *Anopheles* (flies) would appear to be absent during the dry season in Freetown, though they were actually there as proved by the eggs that were promptly laid in the experimental pools. At the Houssa Cantonment and elsewhere the flies were numerous about native dwellings during the dry season, although this lasted several months and the breeding-places were situated at the distance of a mile. The flies persist in the houses and lay their eggs as soon as the rains give rise to pools in the immediate vicinity.

As shown above we have scarcely any exact figures with regard to the number of *Anopheles* encountered in malarious localities. It is usually stated that mosquitoes are numerous without any reference to

the species. We are able however in a general way to conclude from the writings of various authors that *Anopheles* may be very numerous at times. In our search for *Anopheles* in England, when we exclude the observations made by the entomologists who have favoured us with information, we confined ourselves to looking for larvae. At an early date in our investigations we found that this was by far the most rapid method of detecting the presence of these insects in a given district. It is quite evident from what we have found that *Anopheles* are much scarcer in England than in the malarious countries named, for during our search for larvae we were never molested by the winged insect, although the larvae abounded in certain waters. Mr Theobald, who has given especial attention to the *Culicidae*, wrote to one of us that he had never known *Anopheles* to bite in England, and this statement in a manner corroborates what we have noticed in the field. We made no attempts at collecting the flies by means of a net, and we never noticed a single winged insect in the open, though we in a few instances succeeded in capturing them in dwellings. Though we might very well have found the flies if we had searched more closely, the mere fact of our never seeing a single fly in the open country is distinctly suggestive. Though our observations show that *Anopheles* persist in districts formerly malarious, we have noted their presence in localities where as far as we can ascertain there never was malaria. Owing to the greater expanse of suitable waters in the low land which was formerly malarious we believe that *Anopheles* are still most numerous in those regions. That the English *Anopheles maculipennis* is just as fond of human blood as its continental *confrères* has been amply proved by experiment during July and August. Our investigations show that Grassi's generalizations are incorrect. *Anopheles* may occur in non-malarious regions, and consequently his whole deduction whereby he excludes all other blood-sucking insects from being hosts of the parasites of malaria on the ground of their more general geographical distribution, is proved to be premature and fallacious. It is quite possible that there may be other hosts than the *Anopheles* already experimented upon, and it remains necessary to exclude these by actual experiments, such as Grassi himself has made with various species of *Culex*, whereby he has proved that these species are not suitable hosts. It might be added here that Howard (1900, p. 18) suggests the advisability of thus experimenting with the genera *Psorophora* and *Megarhinus*, and there seems no other way of excluding other blood-sucking insects except by experimenting with all of them.

The investigations here recorded were undertaken primarily with the view of determining whether or no *Anopheles* still existed in previously malarious districts, and if this was the case we desired if possible to further investigate whether there was any interrelation between the more or less remote date of the disappearance of malaria and the present numerical distribution of *Anopheles*. For this reason the distribution of *Anopheles* and ague respectively as figured in the accompanying maps may seem to coincide more closely than it should. In other words, we have by no means searched as diligently in regions where malaria was absent as we have searched in those where malaria prevailed. There are many parts of Great Britain which still remain to be investigated. The task is however so extensive that it can only be accomplished with the aid of numbers of trustworthy investigators interested in the subject. Another matter has to be considered in examining the map relating to *Anopheles*, and that is that in certain places where the collectors have resided for some time the positive findings are much more closely aggregated, a fact which would make it appear as if the *Anopheles* were more numerous about the particular region. When as in the table it is noted that many larvae were found in a given pond, it does not follow that *Anopheles* were numerous in the particular region because the pond may be the only breeding-place for a large area. We know that there are more suitable waters for the development of *Anopheles* in the low-lying parts of England, where malaria used to prevail and where of necessity *Anopheles* is most numerous to-day. The fact that we have observed *Anopheles* in out of the way places has also its significance, for it explains how malaria might arise without the introduction of these insects provided an infected man visited the district, other conditions were suitable, and the insects sufficiently numerous. It will also explain how in certain years malaria has been known to spread beyond its endemic centres to regions previously free from the disease. Enough has been done to show that *Anopheles* are to be found in regions where there is no reason to believe malaria was ever endemic.

Regarded from the point of elevation above sea-level we find that we have collected *Anopheles* at 74 places at or near sea-level (50 feet or under), at 46 places below 100 feet, 32 places between 100 and 200 feet, 12 places between 200 and 300 feet, 4 places between 300 and 400 feet, 1 at 400 feet, 2 at 500 feet, and 2 at 600 and 700 feet respectively.

When we come to consider the nature of the water in which the larvae occur we find that they have been captured 41 times in ponds

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(9 times together with *Culex*), in ditches in which the water had a scarcely perceptible flow 36 times (3 times with *Culex*), in ditches containing flowing water 15 times, in rivers and canals (Derwent, Orwell, Ouse, Thames, Lee, Cam, Mole, Ex, etc.) 26 times, and 3 times in the backwaters of rivers. Larvae were moreover captured 6 times in brackish water (twice in ditches, four times in pools), twice in waterlogged boats (once with *Culex*), and twice in stone troughs (once with *Culex*). In only 9 cases was it noted that the water was dirty. Altogether *Anopheles* larvae were found 14 times together with those of *Culex* and 10 times with fish. It might be of interest to add here that one of us (G. H. F. N.) found the larvae of *A. maculipennis* 3 times in Germany in August and September: at Godesberg on the Rhine in a fountain basin together with *Culex* and apparently in the absence of algae; at Treves on the rapidly flowing Mosel, in a dirty puddle, containing *Ulva*, beside the river; near Mölln in Mecklenburg in permanent pools containing *Spirogyra* (no malaria in this district for 25 years as at Godesberg). As in other countries the larvae in England are usually found in water containing algae, usually *Spirogyra*, frequently *Ulva*. They have also been found in water covered by a moderate amount of *Lemna* (duckweed), whereas, as Grassi noted in Italy, they are absent when the water is entirely covered with this plant. At other times the larvae were found in accumulations of aquatic plants which had been torn up from the bottom of rivers and accumulated in little bays and inlets along the banks of the stream. Finally larvae were on several occasions found in waters which appeared to contain no algae, though doubtless they were not entirely absent.

Referring to our notes we find that *A. maculipennis* has been much more frequently found than *A. bifurcatus* and that *A. nigripes* is rare. Of 156 lots of insects collected whose species was determined 123 were *A. maculipennis*, 27 *A. bifurcatus*, and only 6 *A. nigripes*. We have been unable to note any differences in the geographical distribution of these species considered separately.

Methods of Investigation.

In collecting the larvae of *Anopheles* we have found the following simple apparatus fully sufficient. (1) Some wide-mouthed bottles of medium size with cork stoppers; (2) a white enamelled dipper, which can when required be tied with a piece of twine to a light bamboo rod about four feet long; (3) a small pipette with a rubber bulb; (4) small vials containing dilute alcohol, which is subsequently concentrated, serve for the preservation of larvae when it is not necessary to keep them

alive; (5) the collector should be provided with labels, note-book and pencil. The larvae as Grassi has pointed out (1900, June 4) can be distinguished from one another by the character of the setae on the head, as will be mentioned in the following communication. We have found a bicycle an almost indispensable adjunct in collecting. The larvae contained in the bottles, which should be about half filled with water and wrapped in soft cloths placed in a bag on the bicycle-frame, can be transported for several hours on the machine without injury. It was noted that the large larvae and pupae did not withstand the shaking as well as did the smaller larvae, but a sufficient number could always be brought back to Cambridge for breeding purposes. On expeditions lasting a couple of days it is well to loosen the corks occasionally to give the insects fresh air. The use of the white dipper has the advantage of making it easy to quickly detect the eggs or larvae upon the white background, the pipette being used for transferring them to the collecting bottles. Only rarely could larvae be detected by direct inspection of the surface of the water, which in any case is very fatiguing. On account of a similarity existing between the imago of *A. bifurcatus* and that of *A. nigripes* the determination of these species was left to Mr F. V. Theobald, who has very kindly aided us, not only in the matter of identification, but also in generously placing valuable notes at our disposal, these notes being included in our tables. As will be seen in the tables, we are moreover indebted to the following gentlemen for kind and ready aid in our investigation, and we take this occasion for expressing to them our cordial appreciation: Mr Ralph C. Bradley of Moseley, Birmingham; Mr F. G. Binnie of Cambridge; Mr G. H. Carpenter of the Science and Art Museum, Dublin; Mr Eric Gardner, who collected for us in Wales, and has furnished us with Ordnance maps showing the result of his investigation; Dr J. R. Garrood of Huntingdon; Mr Percy H. Grimshaw, F.E.S., of the Science and Art Museum, Edinburgh; Mr H. M. Lefroy of the Imperial Department of Agriculture, Barbados; Mr Claude Morley, F.E.S., of Ipswich; to repeat Mr Frederick V. Theobald, M.A., F.E.S., of Wye in Kent; Mr Alfred Thornley, M.A., F.L.S., F.E.S., President of the Notts. Naturalists Society, and Mr George H. Verrall, F.E.S., of Newmarket. We are indebted to these gentlemen chiefly for data concerning the winged insects. Finally we wish to state that we were very ably assisted throughout our investigation by Walter Mitchell, our Laboratory Assistant, who has shown much enthusiasm and interest in the work to the success of which he has materially contributed.

DISTRIBUTION OF ANOPHELES IN ENGLAND.

In the following table the counties are roughly ordered, beginning with the northern ones and going south. The abbreviations L. or l. and F. or f. signify respectively "larvae" and "fly"; + indicates that attempts to raise the fly failed, or that the larvae died or were lost, and that the species could not be determined. The elevation above the sea was determined by reference to Ordnance Maps both large and reduced (Bartholomew's Tourist and Cyclist's Maps, scale 2 miles to an inch) and are mostly given approximately. The sign - before a number, as in "-100" signifies that the place mentioned is situated at an elevation of 100 feet or under, above sea-level. The letters *b.*, *m.* and *n.* in the column "Species" signify *bifurcatus*, *maculipennis* and *nigripes*. The collector's initials in the last column refer to Dr Nuttall (G. H. F. N.), Dr Cobbett (L. C.), Mr Strangeways-Pigg (T. S. P.) and Walter Mitchell (M.) our Laboratory Assistant. The collecting was all done in 1900. The names of other collectors are given in full.

County	Place	Height above sea in feet	Species	Notes	Collector and date
Yorkshire	Filey	100—200	<i>b.</i>	L. in pond half way down mud-cliff 1 m. S.E. with <i>Culex</i> , also found $\frac{1}{2}$ m. N.W. (flies hatched out)	L. C. 28. viii
			<i>m.</i>	F. (one) caught near Filey	Theobald ix. 1896
	Cayton Bay	50	<i>m.</i>	L. in pond situated as at Filey, with <i>Culex</i> . No algae ¹ . Grass growing up through water, small horse-trodden pools	L. C. 29. viii
	Speeton	40	+	L. in cliff-pond with grassy margins as above, with <i>Culex</i>	L. C. 29. viii
	Robin Hood's Bay	50	+	L. found as above	L. C. 30. viii
	Gristhorpe Bay	50	+	L. found as above	L. C. 1. ix
	Harwood Dale (on the moors between Scarboro' and Whitby)	over 200	<i>m.</i>	L. in pond by a stream in upland grassy valley, with <i>Culex</i>	L. C. 30. viii
	Aysgarth	600	<i>b.</i>	L. absent June and July (water cold), present in Sept. (water warmer) in little spring-fed pools overhung by vegetation in rocky river-bed between bridge and middle fall N. side of River Ure. Numerous	L. C. 9. ix
	Jervaulx	300—400	+	L. in large fish-pond with grassy margins. Fair number	L. C. 8. ix
	Flamboro' Head	170	<i>m.</i>	L. in horse-trodden grass-bordered ponds on top of chalk cliffs (covered with glacial drift) between Danes Dyke and Light House. (a) Small pond near cliff edge $\frac{1}{2}$ m. N. of Light House, many larvae. (b) Roadside pond 1 m. N. of Flamboro' Village, many. (c) Roadside pond inside Danes Dyke	L. C. 31. viii 5. ix
	Buckton	250	<i>m.</i>	L. together with <i>Culex</i> , both numerous at head of large pond with grassy horse-trodden margin	L. C. 31. viii

¹ That is none such as *Spirogyra*, *Ulva*, etc., in quantities visible to the naked eye.

County	Place	Height above sea in feet	Species	Notes	Collector and date
Yorkshire (cont.)	Muston (on road to Filey)	150	<i>b.</i>	L. in horse-pond as before. Flies raised in laboratory	L. C. 3. ix
	Muston-Hun- manby road	150	+	L. in two horse-ponds as before	L. C. 3. ix
	East Heslerton	100—200	<i>m.</i>	L. in little village pond in middle of road. No weed nor grass	L. C. 4. viii
	Causton	140	<i>m.</i>	L. in stone trough. Slow running water, algae. (Fly raised)	L. C. 8. ix
	Village Causton-	110	+	L. in little grassy roadside pond	L. C. 8. ix
	Seamer road				
	Sessay (Vale of York)	50—100	+	L. in grassy horse-pond	"
	Baldersby	100	+	Same as above	"
	(Vale of York)				
	Hertford	90	<i>b.</i>	Many l. in one ditch, few in another (flowing water). None in grassy puddles by chalk- stream	L. C. 4. ix
	River Marsh near Muston				
	Yedingham	75	+	L. in River Derwent and communicating ditch. Slow flowing water. Fair number	"
	Abbey				
	West Ayton	100	+	Very many l. in Old Castle fish-ponds, near River Derwent. (Maximum 12 per "dip.") Pond overgrown with flags, grassy borders, algae. No l. in river close by, nor at Forge Valley	L. C. 6. ix
	Howden	- 50	+	L. in slow flowing ditch (duckweed) 1 m. W. of Howden on road to Heming- borough. Fair number, i.e. 1-2 per "dip"	G. H. F. N. 11. viii
	Hull	- 50	+	L. found along grassy borders of rapidly flowing Barmston Drain, at Newland. Broad deep stream. Clumps of floating green weed and Spirogyra along banks. One l. per five dips. Also Culex	G. H. F. N. 12. viii
Derby	Beauchief Abbey	400	+	No particulars	Lefroy
Lancashire	Carr Hoo	- 50	+	Very few l. in cattle-pond	M. 10. ix
	Walmer- Bridge	- 50	<i>m.</i>	Very few small l. with those of Culex in cattle-pond	"
	Preston	- 100	<i>m.</i>	Only three l. caught in pond by canal on road to Fullwood	M. 11. ix
	Kirkham	- 50	<i>m.</i>	Only two l. caught in duck-pond	"
	Lytham	- 50	<i>m.</i>	A few small l. in ditch on road to Kirkham	"
	Morecamb (and road to Lancaster)	- 50	<i>m.</i>	L. fairly numerous in some ditches	M. 12. ix
	Bay Horse	- 100	<i>m.</i>	L. fairly numerous in ditch, flowing water, in one place only. Algae	M. 14. ix
	Catterall (near Garstang)	- 100	<i>m.</i>	L. plentiful in ponds at Catterall, none at Garstang in ponds and streams. (Fly raised)	"
	St Ann's-on- the-Sea	- 50	<i>m.</i>	One l. caught	Swainson 1900 (comm. by Theobald)
Cheshire	Aldford (near)	- 100	<i>b.</i>	L. in one pond only	M. 9. ix
	Mickle Trafford	- 100	<i>b.</i>	L. in roadside ditch	"

County	Place	Height above sea in feet	Species	Notes	Collector and date
Lincolnshire	Cadney, Brigg	- 50	<i>b.</i>	One ♀ f. caught	Grimshaw v. 1898
	Gainsborough (near)	- 50	<i>b.</i>	L. very numerous in roadside ditch between Morton and Walkerith Ferry. One <i>Culex</i> larva. No algae, only Lemna	G.H.F.N. 12. viii
	Boston	near sea-level	+	(a) Few small l. in Maud Foster Canal, Horncastle Road, algae, water dirty (b) Same in 45-foot Canal	G.H.F.N. 13. viii
	Grantham	- 200	<i>m.</i>	F. caught	Thornley
	Long Sutton	- 50	<i>m.</i>	L. and pupae numerous in ditches both E. and W.	M. 21. vii
	Gedney	- 50	<i>m.</i>	L. numerous in ditches all along road to Eye Green	"
	Holbeach	- 50	<i>m.</i>	" " " "	"
	Spalding	- 50	<i>m.</i>	" " " "	"
	Cowbit	- 50	<i>m.</i>	" " " "	"
	Crowland	- 50	<i>m.</i>	" " " "	"
	Eye Green	- 50	<i>m.</i>	" " " "	"
				(Here <i>Culex</i> but no <i>Anopheles</i> -l. found in a water-butt)	
Nottingham- shire	South Leverton	- 100	<i>m.</i>	One ♀ f. caught. (Highest land 280 ft above sea)	Grimshaw 10. ii. 1898
			<i>m.</i>	F. occurs sparingly on the windows of the house: often during winter. Caught two ♀ 10. ii and 7. iv. 1898 and one ♂ ix. 1900	Thornley 1898—1900
Norfolk	King's Lynn	sea-level	<i>m.</i>	(a) L. plentiful in large and small ditches near station. Water deep, cool, algae present. Larvae mostly small (b) Eggs l. and pupae very numerous in brackish pool to E. of River Ouse estuary. Contained algae chiefly <i>Ulva</i> (<i>Tetraspora</i>)	G.H.F.N. 14. vii
	Norwich	- 50	<i>m.</i>	F. caught	Theobald iv. 1897
	Cromer	- 100	<i>m.</i>	One f. caught	Theobald ix. 1897
	Diss	- 100	<i>m.</i>	F. caught	Verrall 19. vii and 21. viii
	Acle	- 50	<i>m.</i>	L. numerous in roadside ditches (fen drains) containing <i>Spirogyra</i>	G.H.F.N. 3. viii
	Billockby	- 50	<i>m.</i>	ditto	"
	Great Yarmouth	sea-level	<i>m.</i>	L. numerous in ditch near Volunteer En- campment containing <i>Spirogyra</i> . (Flies raised)	"
	St Olaves	near sea-level	<i>m.</i>	Ditch near station. Water brackish and contained algae. (Fly raised)	"
Suffolk	Newmarket	- 200	<i>m.</i>	Five to six f. caught each year in collector's house	Verrall 1. i to 27. xii. 1899
	Mildenhall	- 50	<i>b.</i>	F. caught	Verrall 8. ix ...
	Halesworth	- 50	<i>m.</i>	L. in ditches containing <i>Spirogyra</i>	G.H.F.N. 4. ix
	Southwold	- 50	<i>m.</i>	Fly (one)	Morley 1. viii

County	Place	Height above sea in feet	Species	Notes	Collector and date
Suffolk (cont.)	Wickham	- 50	<i>m.</i>	L. in ditches containing Spirogyra	G.H.F.N. 4. ix
	Market Southwold	- 50	<i>m.</i>	F. caught several times in bedrooms during the night	Morley 1. viii. 1900
	Bury St Edmunds	- 100	<i>m.</i>	L. very plentiful in algae-containing ditches near station on the right coming from Cambridge	G.H.F.N. 28. vii
	Blakenham	- 50	<i>m.</i>	L. plentiful in River Orwell alongside Mill, amongst algae near bank. Deep, slow flowing clear water—river above and below looked likely	"
	Felixstowe- ferry (near)	almost and at sea-level	<i>m.</i>	(a) Few l. found with Culex in dirty water of small pool containing algae (b) Many l. in clear ditches (Spirogyra) near River Deben	G.H.F.N. 29. vii
	Buckleston's Mill (near Newbourn)	- 50	<i>m.</i>	L. numerous in clear, deep, slowly flowing water of mill-pond	"
	Foxhall	- 50	<i>b.</i>	F. "swept from nettles on the side of a stagnant pool." One ♂	Morley 19. v. 1900
Cambridge- shire	Cambridge	- 50	<i>m.</i>	F. caught	Theobald ii to vi 1889—1894
	"	"	<i>m.</i>	(a) Shallow ditch on Grantchester Meadow near University bathing house. Five l. caught, together with small fish, tadpoles, Assellus, etc. A week later no l. there and did not reappear later	G.H.F.N. 10. v. 1900 kindness of E. Bles.
	"	"	<i>m.</i>	(b) L. in ditch leading to mill-pit on Sheep's Green, algae, fish, water shallow. 150 l. caught. 19. vii. caught 191. In August ditch dry	G.H.F.N. 29. vi
	"	"	<i>m.</i>	(c) One ♂ f. caught in outhouse at Pathol. Laboratory	G.H.F.N. 7. vii
	"	"	<i>m.</i>	(d) Three ♀ f. caught in two private houses on Adams and Cranmer Roads	G.H.F.N. Sept. Oct.
	"	"	<i>m.</i>	(e) Two l. in River Granta near Newnham	M. 20. x
	Cambridge- Histon road	- 50	<i>m.</i>	L. in small pond, bordered with grass and rushes, near road, $\frac{3}{4}$ m. from corner of Huntingdon Road. Five l. and one pupa caught, also Chironomus, water not pure	G.H.F.N. 24. vi
	Cambridge- Ely r.r.	- 50	<i>m.</i>	22 l. caught in pools near railroad just N. of River Cam	M. 19. vii
	Girton (near)	- 50	<i>m.</i>	L. in ditch crossed by bridge $3\frac{1}{4}$ m. from Cambridge on Via Devana. Water two inches deep, stagnant, muddy, no green algae. Only two small l. and two Culex caught	G.H.F.N. 21. vii
	Waterbeach	- 50	<i>m.</i>	(a) 53 l. caught in ditch (b) L. in ditch near railway crossing, fair number	M. 6. vii L. C. 22. viii
	Clayhithe (near Water- beach)	- 50	+	(a) L. in fish-pond containing small pike, and inch-long roach and dace, algae (Ulva). Very many l. 6-10 per dip the maximum (b) L. in dead water at bend of river, weed, many l. (12 a dip or none, irregularly distributed). None in ditch alongside of river though water suitable	L. C. 22. viii

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County	Place	Height above sea in feet	Species	Notes	Collector and date
Cambridge- shire (<i>cont.</i>)	Ely	- 50	<i>m.</i>	212 l. caught in long ditch E. and close to Ely station on road to Newmarket. Water at midday 24° C., green algae, slow running peaty tinted water, 1 foot deep, no fish, Assellus plentiful	G.H.F.N. 14. vii
	Littleport	- 50	<i>m.</i>	91 l. caught in small roadside ditch filled with much yellowish-green, dead weed and algae. Water 24° C. (Littleport is 6 m. N. of Ely)	G.H.F.N. 14. vii
	March	- 50	<i>m.</i>	(a) L. in shallow pools along almost dried ditch on high road to Wisbech, few with much Culex (b) L. numerous in ditch where road turns N. to Wisbech (c) L. in ditches all along road to Sutton	M. 21. vii
	Wicken Fen	- 50	<i>m.</i>	F. caught	Verrall 11-19. vii
			+	L. found in water-logged boat in ditch, no algae. Very many, also Culex. Few in ditch	L. C. 19. viii
	Wicken Village	- 50	+	Very few l. found with countless Culex in very dirty, almost dry, ditch in middle of village. Six rain-water tubs only contained Culex	L. C. 19. viii
	Burwell	- 50	<i>b.</i>	F. caught	Verrall 5. v
	Exning	- 100	<i>b.</i>	F. caught	Verrall 11. viii
Huntingdon	St Neot's (and district)	- 100	<i>m.</i>	F. abundant in spring time. Caught during a number of years	Theobald (1900)
	Alconbury Hill (near and N. of Huntingdon)	- 100	<i>b.</i>	♂ and ♀ f. caught by lamplight in house and sent to us. Species determined by Theobald	Garrood 12. viii 1900
	Fen Stanton	- 50	<i>m.</i>	L. very numerous in broad ditch 3 feet deep, flowing water containing algae (at 6 mile post from Godmanchester)	G.H.F.N. 21. vii
	St Ives	- 50	<i>m.</i>	L. in small ditch estuary into Ouse on Houghton path	,,
Bedfordshire	Sandy (near)	- 100	<i>m.</i>	On road to Biggleswade. A few small l. with many of Culex, found in a ditch fed through overflow from small stream, in which Anopheles were plentiful, but Culex absent	M. 4. viii
	Bedford (near)	- 100	<i>m.</i>	On road from Sandy, 3 miles from Bedford. L. in small stream	,,
	Bedford	- 100	<i>m.</i>	A few small l. in River Ouse (in patches of algae) along town promenade	,,
Hertford- shire	Broxbourne	- 100	+	L. in backwater of River Lea near railway station	M. 5. viii
	Rye House	- 100	+	One l. caught in ditch near toll-house	,,
	Bishops Stortford	200-300	<i>m.</i>	L. found in stream	,,
Essex	Lexden (near Colchester)	- 100	<i>m.</i>	Very few small l. in stream	M. 2. ix

County	Place	Height above sea in feet	Species	Notes	Collector and date
Essex (cont.)	Bottle End (near Colchester)	- 200	m.	A few small l. in pond (Bottle End is on road to Maldon)	M. 2. ix
	Heybridge (near Maldon)	- 50	m.	L. plentiful in ditch containing brackish water	,,
	Rochford and near the River Crouch	- 50	m.	L. fairly numerous in a stream, the Cam. Small l. plentiful, in brackish pond	,,
	Pitsea	- 50	m.	L. plentiful in ditch and pond	M. 3. ix T. S. P. 18. ix
	West Tilbury	- 50	m.	Very few l. in a ditch	M. 3. ix
	Corringham	- 50	m.	Many l. in ditches situated $\frac{1}{2}$ mile from church. Water brackish	T. S. P. 18. ix
	Onger (near to, on road to Brentwood)	200—300	m.	L. fairly numerous in pond	,,
	Matching Green and Hatfield Heath	200—300	m.	L. fairly numerous in roadside ditch	,,
	Bulvan	- 50	m.	Very many l. in ditch in Fenland. F. raised	,,
	Vange	50	+	L. plentiful in pond	,,
	Ockenden	50—100	+	A few l. in pond	T. S. P. 19. ix
	Hornchurch	50—100	+	,,	,,
	Rainham	- 50	+	L. in marsh ditches	,,
Sussex	Hastings	- 100	m.	F. caught	Theobald iv and ix 1883—1890
	Rye	- 50	m.	,,	Theobald v. 1885
	Laughton	- 100	m.	,,	Verrall 17. iv ...
	Plashet Woods	500	b.	,,	Verrall 3. vii ...
	Barcombe	- 100	b.	,,	Verrall 21. vi ...
	Lewes	- 100	m.	,,	Verrall 2 & 8 ii ... 15. x & 6. xi
	Seaford	- 50	+	No particulars	,, ... Lefroy
Kent	Wye	- 150	m.	F. abundant in January to May and again in autumn	Theobald 1895—1900
			n.	Rare, only one f. caught	Theobald 1898
	Folkestone	- 150	m.	One f. caught	Theobald iv ...
	Tenterden	- 200	m.	F. caught	Theobald iv. 1899
	Canterbury	- 100	m.	,,	Theobald iv. 1898
	Gravesend	- 50	m.	Many l. found in one ditch only	M. 6. ix
	Queenboro'	- 50	m.	L. fairly numerous in a pond	M. 5. ix
	Queenbridge	- 50	m.	A few l. in ditch, water not clear	,,

County	Place	Height above sea in feet	Species	Notes	Collector and date
Hampshire	Lyndhurst	- 150	<i>n.</i>	(In the New Forest) comparatively rare. F. caught	Verrall 10. vi to 20. viii
	Lymington	- 100	<i>b.</i>	F. caught	Verrall 22. vi.....
	Odiham	200—300	<i>m.</i>	Many l. in ditch	T. S. P. 31. viii
	Fareham	- 50	<i>m.</i>	Very many l. in pond	"
	Portsmouth	near sea-level	<i>m.</i>	F. caught end of August in a garden among thick ivy bushes. L. eggs and pupae found in small stone tank fed occasionally from tap and containing algae. Together with <i>Culex</i>	Bassett- Smith (see biblio.) 1900
	Sandford (Isle of Wight)	- 200	<i>m.</i>	L. in pond	T. S. P. 31. viii
Surrey	Chertsey	ca. 40	<i>m.</i>	Four l. found after long search along grassy marshy margin of a little Thames back- water	L. C. 25. ix
	Weybridge	38 and 200	<i>m.</i>	A few l. along bank of Thames opposite Docket Point. Also at Weybridge Brick- fields in St George's Hills, in a little puddle in clay with sparse vegetation about a fallen branch. Fair numbers. One ♀ f. caught in a house on the common	"
	Esher	48	<i>m.</i>	F. common	Theobald iv. 1888
			+	L. caught in River Mole	L. C. 18. ix
	Wisley	- 100	<i>m.</i>	L. fairly numerous in small pond at head of Hut Pond. Water covered with brown, floating weed	L. C. 20. ix
	Chobham	100—200	<i>m.</i>	L. fairly numerous in small pond, "The Springs," situated in large expanse of heather. Small rushes and moss on bank, water covered with brown floating weed. None found in similar "Gracious Pond" a ½ mile away	L. C. 21. ix
	Peaslake (Greensand hills near Leith Hill)	407	<i>m.</i>	A few l. and pupae found in little rapid, grassy margined, clear stream at roadside in village	L. C. 23. ix
	Friday Street (Leith Hill)	500	<i>m.</i>	L. fairly numerous along margins of large pond	"
	Kingston- upon-Thames	- 50	<i>m.</i>	F. abundant	Theobald iv. 1887
	Richmond	50—100	<i>b.</i>	(a) A few l. in one (middle pond) of the Pen Ponds in Park. Absent in upper pond though conditions similar: margins mossy, short rushes (b) L. fairly numerous in a similar pond in Park near Ham Gate (c) Many pupae and few l. in a little grass- bordered stream near Roehampton Gate. (Flies raised)	L. C. 18. ix
	Godalming	- 200	<i>m.</i>	F. recently caught, one ♀	Theobald 1900
	Denmark Hill London, S.E.	- 50	<i>m.</i>	F. caught	Verrall 23. x.....

County	Place	Height above sea in feet	Species	Notes	Collector and date
Middlesex	Ladbroke Grove, London, W.	- 150	<i>m.</i>	F. caught	Theobald x. 1900
	London, N.W.	- 250	<i>m.</i>	" "	"
Oxfordshire	Oxford	190	<i>m.</i>	A few l. found after long search in ditches in Port Meadow. Many little fish present	L. C. 12. ix
	Culham	150	+	Anopheles and Culex l. plentiful in grassy horse-trodden ditch. One Anopheles l. caught in lock-cut	L. C. 13. ix
	Clifton	140	<i>m.</i>	A few l. in flowing water in ditch communicating with river	L. C. 13-14. ix
	Hampton	105	<i>m.</i>	L. fairly numerous in a small shallow bay in River Thames one mile below W.	L. C. 17. ix
	Whitchurch			Plenty of algae	
	Mapledurham	100	<i>m.</i>	Very many l. in middle of River Thames between island and shore. Slow flow, flags and much Ulva	"
Warwickshire	Sutton Coldfield	300-400	<i>b.</i>	F. caught in "a Park of 2000 acres with several streams and pools and boggy ground." Nine species of Culicidae, including Anopheles, caught. A. bifurcatus, not uncommon, caught in collector's garden near small pools in May, June, Aug.—Oct.	Bradley 1891, 1894, 1897
Herefordshire	Tarrington	200-250	<i>b.</i>	One f. ♀ caught ("probably on damp marshy ground")	Yerbury 1. v. 1899
Buckinghamshire	Hurley (opposite)	90	+	Very many l. just below the big weir in floating weed and Spirogyra. Little water flowing over weir	L. C. 18. ix
	Bletchley	200-300	<i>m.</i>	L. in little grassy margined lake containing small fish, swans and ducks. Fly raised	L. C. 12. ix
Berkshire	Sandford	170	<i>m.</i>	(a) Few l. above weir among weeds and rushes, on both sides, as also in neighbouring ditches	L. C. 12-13. ix
				(b) Plentiful below weir. Much algae	
	Day's Lock	130	<i>m.</i>	L. plentiful in water-logged punt in weir stream	L. C. 14. ix
	Cleeve	115	+	Few l. in ditch (rushes and flags) communicating with river. Water flowing	L. C. 15. ix
	Streatley	110	<i>m.</i>	Few l. in over-shadowed ditch on Mill-Island fed by river-water only at flood-times. None found in backwaters after long search	L. C. 16. ix
	Hambledon Lock	- 100	<i>m.</i>	L. fairly numerous just below lock on Berks side in open River Thames, amongst floating debris and weed	L. C. 17. ix
Dorsetshire	Creekmoore (near Poole)	50-100	<i>m.</i>	Very many l., boggy	T. S. P. 1. ix
	Tolpiddle	50-100	<i>m.</i>	L. fairly numerous in farmyard pond with Culex. Water not clear. No drainage into pond	"

County	Place	Height above sea in feet	Species	Notes	Collector and date
Dorsetshire (<i>cont.</i>)	Axminster	50—100	<i>b.</i>	L. fairly numerous in roadside pond	T. S. P. 2. ix
	Yeovil	100—200	<i>m.</i>	L. fairly numerous in drain ditch	T. S. P. 7. ix
	Polsham (near Wells)	50—100	<i>m.</i>	L. plentiful in drain ditch	T. S. P. 6. ix
	Netherbury near Beaminster	100—200	<i>m.</i>	L. caught (species identified by Prof. L. C. Miall)	Lefroy ¹
Somerset- shire	Taunton	50—100	<i>m.</i>	L. plentiful in mill backwater	T. S. P. 5. ix
	Bridgewater	— 50	<i>m.</i>	L. plentiful in ditch in the town	"
	Weston- super-Mare	50—100	<i>m.</i>	Many l. in pond	"
	Bristol	50—100	<i>m.</i>	L. in pond in waste ground near colliery on railroad	T. S. P. 6. ix
Devonshire	Teignmouth	— 100	<i>m.</i>	Two f. caught in June	Theobald 1884
	Cornwood	300—400	<i>m.</i>	F. caught	Verrall 1 Sept. ...
	Slapton-Leigh	200—300	<i>b.</i>	F. caught	Verrall 8 Sept. ...
	Ugbrooke (10 m. S.W. of Exeter)	— 300	<i>n.</i>	F. caught	Verrall 20. viii ...
	Barnstaple	— 50	+	Very few l. in ditch near railroad	T. S. P. 6. ix
	Exeter	50—100	<i>m.</i>	Few l. in River Ex, near St David's station	T. S. P. 5. ix
Cornwall	Penzance	— 200	<i>n.</i>	F. caught	Theobald 1900

¹ Letter to A. E. S., 22 Oct. 1900, wherein no date is given regarding when they were collected, though this was done of late years.

ISOLATED DATA RELATING TO THE DISTRIBUTION OF ANOPHELES IN
WALES, SCOTLAND, AND IRELAND.

WALES.

County	Place	Height above sea in feet	Species	Notes	Collector and date
Carnarvon- shire	Criccieth	- 50	<i>m.</i>	A few f. caught in September	Theobald 1895
	Beddgelert	- 200	<i>n.</i>	"Beaten from a shady garden in front of the Goat Hotel"	Theobald ix. 1900
Glamorgan- shire	Oxwich village ($\frac{1}{4}$ mile from)	sea-level	<i>m.</i>	One l. caught in stream running through marsh, and containing many prawns	Gardner 25. viii 1900

SCOTLAND.

Lanarkshire	Possil Marsh (N. of Glasgow)	100	<i>b.</i>	Several f. caught ♂ and ♀ (species identified by Theobald)	Binnie 7. ix. 1875
Inverness- shire	Nethy Bridge	ca. 700	<i>b.</i>	F. caught. One ♀ 22. vi, and two ♂ 24. vi, on damp, swampy ground. Two ♀ 15. vi and 9. vii, on windows of hotel verandah, <i>Culex pipiens</i> being very troublesome in the evening	Yerbury 1900
Sutherland	The Mound (Junction for Dornoch)	sea-level	<i>b.</i>	F. caught. One ♀ 8. viii, two ♂ 4 and 10. viii, on damp, swampy ground	„
Aberdeen- shire	Torphins	- 300	<i>m. and b.</i>	F. caught. 18 specimens sent to British Museum by	Dr M. J. Wright (comm. by Theobald 1900)

IRELAND.

Dublin	Harold's Cross (suburb of Dublin)	- 150	<i>n.</i>	One ♀ f. caught in Sept., now in Science and Art Museum, Dublin	Carpenter 1899
Down	Hollywood	- 200	<i>m. b. p.¹</i>	F. caught F. caught F. caught. The author notes "probably a small variety of <i>A. bifurcatus</i> "	A. H. Hali- day (Ent. Mag. vol. i. p. 148 1833)

¹ plumbeus.

[illegible]

5

The Former Distribution of Ague
in
ENGLAND



The Former Geographical Distribution of Ague in England.

"And danger like an ague subtly taints even then when we sit idly in the sun."

Troilus and Cressida, Act III. sc. 3.

The history of malarial disease in England almost up to the beginning of the present century is involved in much obscurity. We read of epidemic after epidemic sweeping over this country destroying in its course sometimes half the population of a town. Many of these epidemics were known as the "plague ague," or by some other name which suggests a malarious origin. But Creighton¹ warns us against supposing that the word ague, as used in bygone times, had this significance. Originally he tells us it meant simply an acute illness, being the "adjective of *febris acuta* used as a substantive." In Ireland it was applied until a comparatively recent period to the indigenous typhus of that country. Even the terms tertian, quartan, and the like, as used by old writers, are according to him misleading, and seem to have been borrowed from classical writers, and applied inaptly to the continued fevers of this country. (Creighton, vol. I. p. 411, vol. II. p. 304.) On the other hand Sir Joseph Fayrer², writing of the former prevalence of malarial diseases in this country, tells us, that we have lost two Kings and a Queen, a Cardinal, a Lord Protector, and many other great people from this cause. Creighton however says that "the ague of which Cromwell died in the autumn of 1658 was one of those which raged all over England from 1657 to 1659 so extensively that the country was one hospital," and implies that this was not a malarial epidemic; and he states that "the malarious parts of England have been tolerably well defined at all times, and at all times the greater part of the country was as little malarious as it is now."

Whatever views may be held as to the kind of disease which caused these pestilences, there can be no doubt at all that ague was once very prevalent in certain parts of this country.

In England, malarial disease seems to have been endemic only in the low-lying, ill-drained swampy districts where there was abundance of stagnant or slowly flowing shallow water. Among such places, the principal were the Fens of Cambridgeshire, Lincolnshire, and the sur-

¹ Creighton, C., *A History of Epidemics in Britain*. Cambridge (University Press), 1891 and 1894.

² Sir Joseph Fayrer, *Trans. of the Epidemiol. Soc. of London*, n. s. Vol. I. p. 20, 1881—1882.

rounding counties, the marshes on either side of the estuary of the Thames in Kent and Essex, the marshes of Romney and Pevensey on the south coast, and those around Bridgewater near the Bristol Channel. In such places malarial disease was never absent, but there is good reason to think, as we shall presently show, that it differed greatly in prevalence and severity in different years and occasionally spread thence over the neighbouring country.

There is much difficulty in tracing the earlier history of the disease, owing to the very imperfect knowledge of fevers in general and to the imperfect diagnosis which then prevailed. Conscious of the fallacy of attributing to malaria all diseases which were formerly called by the name "ague" variously qualified, we have limited ourselves in drawing up the list of places where malaria is known to have been endemic, to evidence derived from modern sources, and chiefly to a "Report as to the Quantity of Ague and other Malarious Diseases now prevailing in the principal Marsh Districts of England," drawn up by Dr George Whitley at the request of, and presented to, the Privy Council in 1863¹, and to Dr Peacock's paper on "The recently prevalent malarial affections²." However imperfect this list may be, and we are conscious that it is no more than a bare outline of the more important districts where malarial diseases were once endemic, we may claim with some confidence that the diseases referred to are true instances of malarial fever.

That ague was once much more prevalent than at the time when the authorities quoted wrote, is to be gathered from these authorities themselves, as well as from older writers. Defoe in "An Account of a Tour through the Eastern Counties of England in 1722" gives a grim picture of the agues then prevalent in the marshes around the mouth of the Thames. And, though the humorous character of his description should warn us to take his statements with a grain of salt, there can be no doubt as to the general truth of his description. He tells us on the authority of "a merry fellow whom he afterwards found fibbed a little," that the men being bred in the marshes and seasoned to the place, did pretty well with it, but they always went into the uplands for a wife, and that the women coming from these parts into the fogs and damp presently changed their complexion, got an ague or two, and seldom held it more than a year, and then the men go to the uplands again

¹ Whitley, G. "Residence in Marsh Districts." *Reports from Commissioners*. Vol. xxviii. 1864, p. 430.

² Peacock, "On Recent Malarious Affections." *Med. Times and Gazette*. Vol. xix, 1859, pp. 399, 453, and 478.

and fetch another, so that it was very frequent to meet with men who had had from 5 or 6 to 14 or 15 wives." And in a more serious vein he goes on to say, that in these places the inhabitants do not hold out as in other countries, and you very seldom meet with very ancient people among the poor as in other places, so that take it one with another not one half of the inhabitants are natives of the place. It is curious to find that when he comes to describe the fens of Cambridgeshire, though he speaks of the "fogs and vapors which so universally overspread this country" he goes on to say "yet notwithstanding this the people, especially those which are used to it, live unconcerned and as healthy as other folk, except now and then an ague which they make light of, and there are a great number of very ancient people among them."

From early times the Fens had the reputation of being unhealthy. Thus Camden speaks of Ely being situated on a marshy soil and in unhealthy air, and says that there is a prodigious fen beginning from the banks of the Roman Granta and extending a great way north as far as the sea. But William of Malmesbury (1100 A.D.) speaks of some parts of the fen, for instance Thorney, being favourable to health and a very paradise, and Dugdale concluded that in the 11th century "the outfalls were clear and open to the sea, and this argueth a greater care in the people inhabiting this flat country in those days than has been for several years since¹."

Passing over the epidemic agues of the 17th century of which Sydenham wrote, for it will be necessary to refer to them again, and coming at once to modern times we find that ague was still very prevalent in London as late as 1859. Peacock (*loc. cit.*) gives the proportion of ague cases to the total number of in- and out-patients treated at St Thomas's Hospital from 1852—1859 as follows:

1852	18·4	per 1000.
1853	23·7	"
1854	16·6	"
1855	12·3	"
1856	20·7	"
1857	40·2	"
1858	46·5	"
1859	(9 months)		56·7	"

He remarks that 56·7 per 1000 in the first 9 months of 1859 is a higher rate than we learn from Sir Gilbert Blane existed when he was physician

¹ Quoted by Miller and Skertchley, *The Fenland Past and Present*. London (Longmans), 1878.

to the hospital, namely 50·01 per 1000 from 1783—1794. In the towns of Huntingdon, Wisbech, and North Aylsham much ague still existed and caused a good many deaths. Peacock gives the following figures showing the proportion of deaths attributed to ague to those from all causes:

PROPORTION OF DEATHS FROM AGUE PER 1000 DEATHS
FROM ALL CAUSES.

	Huntingdon	Wisbech	N. Aylsham
1850		4·5	19·1
1851	7·2	0·0	31·2
1852	11·6	5·7	19·5
1853	34·09	3·4	37·3
1854	7·5	1·05	9·07
1855	7·7	0·0	0·0
1856	5·4	2·94	15·2
1857	12·1	9·1	25·2
1858	2·3	6·4	23·3

From the greater prevalence of ague at North Aylsham than at either Huntingdon or Wisbech, it may be inferred that ague was more fatal at that time in the low-lying lands of Kent bordering on the estuary of the Thames than in the fen country around Huntingdon and Wisbech. And this is in substantial agreement with what Defoe wrote, a century earlier.

When Dr Whitley made his report to the Privy Council in 1864, ague had already much decreased in England. He thus sums up his inquiry: "Intermittent and remittent fevers and their consequences can no longer be regarded as affecting the health of the population in many of the districts in which those diseases were formerly of a formidable character. Thus in Norfolk, Lincolnshire and Cambridgeshire, counties in which those diseases were both formidable and severe, all the evidence, except that furnished by the records of Peterborough Infirmary, and in a somewhat less degree in Spalding, tends to show that they are at the present time comparatively rare and mild in form. In several of the places visited, however, it was stated that accessions take place from time to time, and there was a striking agreement generally as to the year in which those accessions occurred.

"The same holds good for the marshes in Hampshire and for all the places visited on the West Coast north of Liverpool.

"In the south part of Essex, in north and south Kent, in the neighbourhood of Lewes and of Bridgewater, a somewhat greater number of cases occur than in the counties mentioned above, but these are

rarely severe. Tertian is the form usually met with, quotidian less frequently, and quartan appears to be almost unknown. Instead of the well-marked paroxysmal ague (once) so prevalent in the marsh districts, an irregular form has succeeded which interferes but little with the usual occupations of those affected.

"It may therefore be safely asserted as regards England generally that the diseases which have been made the subject of the present inquiry have been steadily decreasing both in frequency and severity for several years, and this decrease is attributed in nearly every case to one cause, improved land drainage.

"Of the districts where malarious influence has of late times decreased, but where there still remains much to be accomplished in order that they shall be rendered free from malaria, the most important are Sheppey, Hoo, Spalding, Hull, New Romney, and Lewes.

"Districts where there has not been in late times much, or any lessening of malarious influence are Huntspill, and the marshes on the banks of the river Swale."

Dr Lionel Beale wrote to one of us (Oct. 20, 1900), stating that he used to see many cases of ague at King's College Hospital from about 1845—1865, coming from the marshes about Woolwich, Purfleet, Plumstead, and the neighbourhood, and he used to hear of many cases in Cambridge and the Fen country. Dr J. F. Payne has also written to us (29 Oct. 1900), as follows: "The distribution of ague in place and time in former centuries is rather obscure. The old writers never seem to have taken the trouble to mention the precise districts where it was most common, except quite incidentally. So while certain districts such as the Fen country, Essex, north of Kent etc. got a reputation for malaria the absence of notices of ague in other places does not prove that it did not occur there. There is very little doubt that in the 17th century and even in the 18th ague was endemic in London. Lambeth marsh was notorious, also Westminster and what is now Pimlico. At the beginning of the century it was equally clear, or at least generally believed,—and Murchison believed, that up to the time he saw out-patients at St Thomas's 1850—60,—there were cases of ague in London, not imported. He told me this himself more than once, but I cannot say he ever brought convincing evidence. When I saw out-patients at St Thomas's there used to be a good many cases of ague, chiefly among hop-pickers who had been in Kent in the autumn and whose ague was often latent until the spring. I heard of cases apparently arising in London but never got definite proof of it. But I strongly suspect

that ague lurked in the south of London until the middle of the century."

The only case of ague known to us arising in England, and in which the parasites were found in the blood, is one of which Dr Burton Fanning of Norwich has told us. The patient was in the Norwich hospital from 11th December 1897. to 5th February 1898. He lived at Acle and had never been out of Norfolk. There was no doubt about the nature of the case and the plasmodium was found in his blood by Dr S. Long. Dr Burton Fanning wrote that this was the only Norfolk case in Norwich hospital for many years, but the doctor at Acle had assured him that he saw one or two bona-fide cases every year. Such cases must however be very rare for Dr R. J. Mills of Norwich has written to G. H. F. N. November 1900, saying that he has heard of no instance of this disease within ten years; he had two cases under his care about twenty years ago. He has drawn our attention to the fact that Lord Nelson suffered from ague during his youth, which was spent in Norfolk. Southey¹ remarks that "ague which at that time (1758—1770) was one of the most common diseases in England had greatly reduced his (Nelson's) strength."

On the borders of Scotland ague was formerly very prevalent; and Graham² writing of the 18th century says that this was the one ailment to which the people were most liable. "Terribly prevalent and harassing this malady proved to the rural classes, for every year a vast proportion of the people were prostrated by it, so that it was often extremely difficult to get the necessary work of the fields performed in many districts." From the Scottish Register we learn that ague was very common about Berwick and at Roxborough in 1715. In the records of the Kelso Dispensary³ for the latter part of the eighteenth century are to be found the annual number of cases treated there. They range from 17 in 1777 to 161 and 103 in 1780 and 1781 respectively, and then gradually fall, and after 1796 do not exceed 10. In 1807 they completely disappeared from the books. These figures not only fix the date of the disappearance of ague from this part of Great Britain, but also illustrate the great variation in the prevalence of the disease in different years.

Ireland has had the reputation of being exempt from ague, the

¹ *Life of Nelson.*

² Graham, H. G., *The social life of Scotland in the eighteenth century* (London 1900), Vol. I. p. 185.

³ Christison, *Edinb. Med. Journ.* 1863, p. 427.

peat-bogs being especially stated to be free from this disease, but Wylde¹ speaks of ague in Ireland and of the occurrence of an epidemic in Dublin in 1805, and says that since that time cases have always been met with in Dublin and its neighbourhood. Dr William Stokes² has drawn attention to an epidemic of ague in Ireland in 1829, but considered it to have been imported from the English fens.

It is evident that in places where malarial diseases were endemic the prevalence of the disease varied greatly at different times. And in certain years assumed the proportions of an epidemic. The most recent example of this is afforded by the years 1858 and 59. And a reference to the following table will show that in these years ague was remarkably prevalent in 16 of the places mentioned there; and these include places so far apart as Hull, the Fens of Cambridgeshire, the Thames estuary, and the marshes around Bridgewater in Somersetshire. It is worth noting that these years were described as being remarkably dry. One can now easily believe that this variation in the prevalence of malaria in different years was associated with a similar variation in the numbers of *Anopheles*; for every entomologist knows how greatly the numbers of certain kinds of insects vary in different years, and it is probable, though not as yet an ascertained fact, that the *Anopheles* undergo a like variation in numbers.

Now that we know that *Anopheles* in England are not limited to the low-lying districts though doubtless more numerous there, it will easily be understood that, in seasons which are very favourable to them, the disease which they transmit may spread from the marshes to surrounding districts where they are usually scarce. Examples of such epidemics have occurred in modern times. Macculloch³ wrote in 1827 of "numerous villages in Lincolnshire, Essex, Sussex and Kent, and indeed almost everywhere, in which the autumn used to pass over with a few insulated cases of fever, having been ravaged by epidemics which might well compare with those of many parts of France and Italy. And in the same manner those fevers have appeared where they were formerly unknown, and even their possibility unsuspected; a fact which in many cases seems to have excited considerable surprise among those who resorted to them as formerly to seek for health. That all these have been cases of marsh fever, and not of typhus as commonly supposed, is incontestable."

Another modern instance of an epidemic of malaria was reported by

¹ Wylde, *Edinb. Med. and Surg. Journ.* 1845, p. 263.

² Cited by Wylde.

³ Macculloch, *An Essay on Malaria*. London, 1827, p. 346.

Haviland¹ to have occurred at Cannington, a little village in Somersetshire in 1858. The place is situated on the border of the alluvial plain of the river Parrett three miles N.W. of Bridgewater. Ague once prevalent here, had given but little trouble in recent years, only 1.5% of the cases admitted to the local dispensary in the previous 12 years having been attributed to this cause. In 1858 the number of ague cases at the dispensary amounted to 19% of the total. And at two of the friendly societies to 29%. In all there were 94 cases in a population of 800. It is notable that this little epidemic should have followed an exceptionally dry winter and spring.

Having shown that malarial disease sometimes spreads as an epidemic from those places where it was always present to others where it was usually unknown, we may now return to the "plague agues" of the time of the Restoration of which Sydenham and others wrote. The nature of these diseases is involved in some obscurity, which can only be removed by comparing the accounts of contemporary writers with those of modern authors, who have had the advantage of being able to base their diagnosis on the presence or absence of the plasmodium. Creighton nowhere definitely states whether he believes these epidemics to have been caused by malarial disease or not, but he frequently implies the latter belief. He thinks that "Sydenham was much influenced by the example of Hippocrates in giving prominence to the intermittent type of fevers" (vol. ii. p. 10). Moreover he remarks that Sydenham "had much to say of agues and intermittents prevalent in town and country, for a series of years, and then disappearing for as long as thirteen years at a stretch. But he does not count these as the agues of the marsh; his single reference to the latter is in his essay on Hysteria, where he interpolates a remark that if one spends one or two days in a locality of marshes and lakes, the blood is in the first instance impressed with a certain spirituous miasma which produces quartan ague, and that is apt to be followed, especially in the more aged, by a permanent cachectic state. If Sydenham had intended to bring all the intermittents of his experience into that class he would not have left the paludal origin of them to a casual interpolated remark....On the other hand he refers the epidemic agues which occupy his pen so much to emanations from the bowels of the earth, according to a theory of his friend Robert Boyle....Sydenham and his learned colleagues were not ignorant of the endemic agues of

¹ Haviland, A., *Journal of Public Health and Sanitary Review* 1858, iv. p. 266 et seq.

marshy localities, but they made little account of them in comparison with the agueish or intermittent fevers which came in epidemics all over England."

That Sydenham was really well acquainted with ague the following extracts from his writings¹ will show.

"All agues begin with shiverings and rigors, succeeded by heat and terminated by sweats....The symptoms decline in proportion as the sweats come on, when these have broken out copiously the fit seems to have gone off. He that was just now sick becomes a healthy man....Soon or late however the paroxysm repeats its attack, the intervals being as follows, for the quotidian twenty-four hours, for the tertian eight-and-forty, for the quartan sixty-two" (vol. i. p. 72). "Intermittents are of two kinds, vernal and autumnal. They may occur indeed at any intermediate period but may for the most part be referred to the months of February and August. True it is that the fevers of the two seasons have some common characters between them....In the meantime I am sure they wholly differ from one another essentially. The vernal ones are always quotidian or tertian, neither long nor dangerous....The autumnal intermittents are very different. They may engender a multiplicity of symptoms, scurries, indurated bellies, dropsies. They may begin as early as June in years when the disease is epidemic. They are either tertians or quartans. In the beginning it is no easy matter to detect their intermittence in the first few days, since they may commence with the superaddition of continued fever. It is also difficult at first, unless you observe with great minuteness to detect anything beyond slight remission of the disease. By degrees however they end in perfect intermission, and take the type that answers to the season of the year" (vol. i. pp. 73—78)².

In these disorders he speaks of Jesuits' bark as his sheet-anchor and in another place he says that Peruvian bark "bears the bell," and he frequently refers to it as the great remedy for intermittents. "When occasion requires," he naïvely remarks, "we give it even to our wives and children"; again, "It has been famous in London for over five-and-twenty years. The disease in question was seldom or never cured by any remedy before it, hence agues were justly called the *opprobria medicorum*" (vol. ii. p. 12). "Since 1664 intermittents had been

¹ *The Works of Sydenham*, by R. G. Latham, M.D. 1850. Sydenham Society, London. Two Vols.

² Compare Thayer and Hewetson quoted later.

nearly banished from London for thirteen years. In 1678 they were again epidemic, and by the end of the summer and beginning of autumn they were pre-eminently prevalent, so much so as to exclude all other diseases from the name of epidemic. First I must note that although quartans were at first most common, tertians or quotidians are the commoner now. In like manner then tertians and quotidians, setting in with chills and shivers, followed by heat, and closing in sweats ended for awhile in complete apyrexia, only attacking the patient again after a stated interval." He goes on to say—and this is one of the passages which Creighton takes as showing that the disease in question was not malarial fever, "Nevertheless they kept this course only until the third or fourth fit, especially if the patient took cordials, kept his bed, and so, as the saying is, added fuel to fire, afterwards they so far assumed a severity foreign to their nature, that instead of an intermission there was only a remission. From this they went on to the type of continued fever, and at length affected the brain and proved fatal to many."

If we compare this with modern accounts we find that all the symptoms which Sydenham mentioned are recognised to-day as those of malarial fever.

Prof. Osler¹ gives the following definition of malarial fever. "An infectious disease characterised by (a) paroxysms of intermittent fever of quotidian, tertian or quartan type, (b) a continued fever with marked remission, (c) certain pernicious rapidly fatal forms. And (d) a chronic cachexia, with anæmia and an enlarged spleen." Osler recognises the following clinical forms:—(1) *Intermittent fever*. This form is characterised by recurring paroxysms of what are known as ague, in which as a rule chill fever and sweat follow each other in orderly sequence. (2) *Continued and remittent forms* of malarial fever, known as bilious remittent fever, and typho-malarial fever. The fever is continuous with remissions more or less marked. Intestinal symptoms are not present. A slight hæmatogenous jaundice may develop early. Delirium usually of a mild type may occur. (3) *Pernicious malarial fever*; (a) the comatose form, in which the patient is struck down with the most acute cerebral disturbance, either acute delirium, or more frequently a rapidly developing coma. (4) *The Algid form*. Characterised by vomiting, intense prostration, and feebleness out of all proportion to local symptoms. (5) *The Haemorrhagic form*, with hæmorrhage from the mucous membranes, and Hæmaturia.

Osler mentions the following types of fever as prevalent in the South of the United States, (1) Typhoid fever, (2) Typho-malarial fever,

¹ Osler, W., *Practice of Medicine*. New York, 1892, pp. 140—156.

(3) Malarial remittent fever, (4) Continued thermic fever. He is inclined to think that, except the last, these fevers will ultimately fall into two classes only, Typhoid and Malaria. The presence or absence of the plasmodium he says must be the criterion of diagnosis.

Since this was written ten years ago much work has been done on malarial fevers, especially in Italy and America. And the presence or absence of the parasite has made it possible to distinguish accurately malarial from other types of fever. And it has been clearly shown that in what is now known as aestivo-autumnal malarial fever, definite intermissions may be absent. Marchiafava and Bignami¹ describe cases of malignant fever often of a sub-continued form, accompanied by coma, delirium, eclampsia, hemiplegia, cerebral irritation, tetanic symptoms or haemorrhages, in all of which the characteristic parasites were found. They sum up their experiences with regard to irregular forms of malarial fever thus: "There is no group of fevers which is naturally and *per se* irregular, but fevers of every class may become irregular." Some types are more or less liable to this change, while in others like the aestivo-autumnal type it takes place very frequently.

Thayer and Hewetson², speaking of aestivo-autumnal fever in Baltimore say that there were many instances where overlapping of the paroxysms caused continued fever. "For instance," they say (p. 66), "one may see a typical summer tertian in the recurrence, while in the original infection the fever was irregular or sub-continued. But it is in the quotidian especially which in the majority of cases are observed to be distinctly intermittent only in the relapses."

These quotations will we think show that the epidemic agues of which Sydenham and others after him wrote may well have been true instances of malarial fever. Very serious pestilences they were. Thus Sir George Baker (quoted by Creighton) describes one of them:

"These agues were first noticed in London in the spring and autumn of 1780, but they infested various parts of England a little earlier. In the more inland countries the agues were often attended with peculiarities extraordinary and alarming. For the cold fit was accompanied by spasm and stiffness of the whole body, the jaws being fixed, the eyes staring and the pulse very small and weak. When the hot fit came on, the spasms abated and ceased in the sweating stage, but sometimes the spasm was accompanied by delirium, both lasting to

¹ Marchiafava, E., and Bignami, A., *On Summer-Autumn Malarial Fevers*. Translated by J. H. Thompson, M.D., New Sydenham Soc., London, 1894.

² Thayer, W. S., and Hewetson, J. (1895), *The Malarial Fevers of Baltimore*. Johns Hopkins Hospital Reports, Vol. v. pp. 3—218, 2 plates and bibliography.

the very end of the paroxysm. This fever had every kind of variety and whether at its first accession it were a quotidian, a tertian, or a quartan it was very apt to change from one type to another. Sometimes it returned two days successively and missed the third day and sometimes it became continual. I am not informed that any died of this fever while it intermitted. It is however certain that many country people whose illness had at its beginning put on the appearance of intermission, becoming delirious sank under it in four or five days." "It is a remarkable fact, and well attested, that in many places, whilst the inhabitants of the high grounds were harassed by this fever in its worst form, those of the subjacent valleys were not affected by it. The people of Boston and of the neighbouring villages in the midst of the Fens were in general healthy at a time when fever was epidemic in the more elevated situations of Lincolnshire. Women were nearly exempt, but few male labourers in the fields escaped it." "The distinguishing character of this fever was its resistance to Peruvian bark; nor indeed was the prevalence of the disease more observable than the inefficacy of the remedy¹." Barker of Coleshill (cited by Creighton) writes of the same epidemic of 1781 as follows:—"This spring that very peculiar, irregular, dangerous and obstinate disease, the Burning—or as the people of Kent properly enough called it the Plague-ague made its appearance, became very epidemical in the eastern part of the kingdom, and raged in Leicestershire, the lower part of Northamptonshire, Bedfordshire, and in the Fens throughout the year...This strongly pestilential disease had such an effect upon them that the complexion of their faces continued for a time as white as paper and they went abroad more like walking corpses than living subjects."

From all this we may conclude that ague, always endemic in the marshes, was wont to spread in suitable seasons from its usual haunts and invaded large areas of this country and to rage so extensively that, in the words of Sir Joseph Fayrer, "England was one large hospital."

We have already referred to the probable association of these epidemics with an increase in the number of *Anopheles*; and it is curious to note that Sydenham held "that when insects do swarm extraordinarily and when fevers and agues (especially quartans) appear early, as about midsummer, then autumn commonly proves very sickly²."

¹ Thayer and Hewetson, as also other authors, state that aestivo-autumnal fever is much more resistant to quinine than are tertians and quartans.

² *Life of Sydenham* by R. G. Latham, Introductory to Sydenham's works already cited, Vol. I. p. xxviii.

THE DISTRIBUTION OF AGUE IN ENGLAND IN THE NINETEENTH CENTURY.

*(Places marked * are excluded from the map for want of space.)**(Authorities with (W) added are cited by Whitley.)*

County	Place	Observation	Date	Authority
Westmorland	Kendal	P. gives returns in Kendal Hospital for 1795—1821 and states 118 cases of intermittent fever occurred among 28,700 patients. In 1798 and 1799, 12 and 13 respectively, 19 in 1809, since which they diminished rapidly, only 6 cases in 8 years, 1813—1821	1795—1821	Proudfoot ¹
Cumberland	Carlisle	Ague not endemic. In 1859 of 2580 patients treated at the dispensary, only three entered as suffering from intermittent disease	1864	Whitley ²
Lancashire	Ulverston	No intermittent disease during 30 years' practice, but had heard from others that a good deal had existed at the beginning of the century	1864	Dickinson (surgeon) (W)
	Garstang	There was some intermittent disease at Eccleston in 1826—1827, but none since	1826—1827	Dr Bell (W)
	Kirkham	Saw much ague prior to 1831, having sometimes 40 cases at one time	ante 1831	Dr Gradwell (W)
Yorkshire	Selby	Severe and frequent, especially in surrounding villages about 1823	1823	Burkitt (surgeon) (W)
	Howden	Common among adults and in schools in 1827. Almost unknown in 1864	1827	The schoolmaster (W)
	Hull	Very common many years before 1848—1863 when an average of 10 in-patients and 10 out-patients at the infirmary annually. The maximum occurred in 1857 and 1858	1848—1863	Whitley
	Patrington	Rare, except among inhabitants of Sunk Island, a low-lying district on the coast about two miles south of Patrington	1864	Dudley (surgeon) (W)
	Goole	Very frequent, but not often severe, in the early years of his practice ca. 1827. No well-marked cases in recent years (1864)	1827	Cass (surgeon) (W)
Lincolnshire	Brigg	Scarcely any, except imported cases	1864	Whitley
	Barton	Ague not endemic since he came to Barton, but currently believed to be common prior to drainage	1837	Morley (surgeon) (W)
	Gainsborough	Scarcely any cases on dispensary books. Dr Mackinder had seen none himself during 10 years	1864	Whitley
	Lincoln	Rare, but a few patients from surrounding districts treated in hospital. 12 cases, 1836—1853. 26, 1856—1859	1836—1859	Whitley
	Horncastle Boston	Little known for many years Had seen much ague about 1819, now scarce, 1864. There was an accession of intermittent disease in 1858, 1859	1864 1819—1864	Whitley Coupland (surgeon) (W)

¹ *Edinburgh Med. and Surg. Journ.* 1822, p. 386.² Whitley, *loc. cit.*

County	Place	Observation	Date	Authority
Lincolnshire (cont.)	Bourne	Declining in frequency and severity. Occasional outbreaks of intermittent and remittent fever	1824—1864	Nicholls (surgeon) (W)
	Spalding	(a) Still frequent, but less severe than formerly	1864	Whitley
		(b) Most frequent in 1808, 1826, 1827, 1858, 1859. His books showed 300 cases in private practice alone	1808—1859	Dr Cammack (W)
		(c) In a school of 85 as many as five absent from ague at a time. Of 75 boys questioned, 11 said they had had ague	1858 1864	Whitley
	Holbeach	Comparatively little ague and that mild tertian. There was an increase in 1859	1859	Dr Harper (W)
Norfolk	Long Sutton	Intermittent fever frequent and severe when he came to Sutton (1829), and again in 1858 and 1859	1829, 1858—1859	Ewen (surgeon) (W)
	King's Lynn Walpole- St Peter Terrington- St John's* Wootton*	Severe ague prevalent in 1844, sees scarcely any cases now (1864)	1844, 1864	Deck
				Kendall (surgeon) (W)
		Extremely rare, though very common about 1814	1814	Rev. Clark (W)
	Norwich	Uncommon, 19 cases of ague treated in Norwich Hospital, 1820—1860	1864	House-surgeon (W)
	Acle	Treated a case of ague from Acle, Dec. 1897 to Feb. 1898, parasites found in blood by Dr S. Long, patient had never been out of Norfolk. The only case admitted "into Norwich Hospital for many years." Doctor at Acle stated he observed 1—2 bona fide cases annually	1897	Dr Burton-Fanning of Norwich ¹
	Wells	Formerly frequent as stated in 1864, but rarely if ever fatal	—	Rump (surgeon) (W)
	Walsingham Heacham near Hunstanton	Some cases in 1859. Otherwise rare	1859	Whitley
Suffolk	Lowestoft Bury St Edmunds	See note about Bluntisham below	ca. 1864	Deck
Cambridgeshire	Peterborough	Gave a table of admissions into infirmary, most of the cases being from surrounding fens	1816—1863	Dr Paley (W)
	Ely	Where there was one case about 1864, there had been 20 about 1859. Ague generally present up to 1864 in three or four cottages in Witchford on the edge of the fen	1859—1864	Muriel (surgeon) (W)
	Wisbech	Formerly common but now scarce	1864	Groom (surgeon) (W)
	Whittlesea	Peacock gives mortality tables (see text)	1850—1858	Peacock
		Saw moderate number of mild cases about 1850 to 1864	1850—1864	Crisp (surgeon) (W)
	Swaffham Bulbeck	Very prevalent in 1823, less frequent subsequently	1823—	Rev. Jennings ²

¹ Letter to G. H. F. N. dated Aug. 1900.² Cited by Miller and Skertchly, *The Fenland Past and Present*, Longmans, 1878.

County	Place	Observation	Date	Authority
Cambridge-shire (cont.)	Bluntisham Willingham Chatteris Newmarket	Mr Deck, chemist in Cambridge, informs us that his father supplied quinine pills for ague in these places, and has shown us a list of testimonials. This note applies to the other places where Mr Deck's name is cited	ca. 1864	Deck
	Waterbeach Cambridge (vicinity)			
Hunting-donshire	St Ives	Saw a good deal of ague about 1834, scarcely any during next decade	1834—1844	Girling (surgeon)
	Warboys	See note above relating to Bluntisham	ca. 1864	(W) Deck
	Little Stukely Wistow			
	Huntingdon	Peacock gives mortality tables (see Text)	1851—1858	Peacock
Bedford-shire	Potton	See note above relating to Bluntisham	ca. 1864	Deck
	St Neots			
Essex	Rochford	Ague formerly extremely common and severe, but gradually decreased	1817, 1864	Dr Grabham (W)
		Very common among the children about 1849	1849	School-master (W)
	Mucking Bulpham Corringham	Still not uncommon though no longer very severe	1864	Corbet (surgeon) (W)
	Maldon			
		The greatest prevalence of late years was in October, 1859. Not $\frac{1}{100}$ of the ague in 1864 that existed 20 years earlier	1859	Tomlinson (surgeon) (W)
	Tilbury	"Tilbury fort has long been regarded as unhealthy, and the troops have of late years been relieved every six months." In 1873 there were 12 admissions for ague among 102 men quartered at the fort during the first six months, and in the same period in 1872 there were 34 admissions for ague among 103 men stationed there	1872—1873	Faught ¹
	Romford	The neighbourhood was peculiarly exempt from ague about 1864, but one or two cases occurred in the town itself in 1859	1859	Deck Davey (surgeon) (W)
	Rainham	Several cases	"	"
Surrey	London	(a) Gives the proportion of cases of ague to total number of patients admitted to St Thomas's Hospital (see Text)	1850—1859	Peacock
		(b) Refers to existence of ague on the Surrey side of London	1847	Hicks ²
Kent	Sheerness	Ague prevailed to an unusual extent during 1858—59 in most of the districts where it was still met with in 1864. It appeared in those years in places, especially elevated ones, where it was previously almost or entirely unknown. It decidedly decreased after 1860	1858—1860	Whitley

¹ Faught, Surgeon-Major J. G., "Report on the Prevalence of Ague at Tilbury Fort." *Army Med. Depart. Report*, 1874, xvi. p. 35 and 1875 (London, 1877), xvii. pp. 212—216. Two plates.

² Hicks (1847), "On Malaria, with a few cases illustrative of its existence on the Surrey side of the Thames." *London Med. Gazette*, n. s. Vol. iv. p. 121.

County	Place	Observation	Date	Authority
Kent (cont.)	Milton	Intermittent disease rather common	1864	Ray (surgeon) (W)
	Faversham	In spring and autumn one-eighth to one-sixth of the children are usually affected with ague The house-surgeon stated, 1864, that there was then scarcely any ague, but it was common and severe when he first came (1838), and in the dry summer of 1859 many severe cases occurred	1838—1859	School-master (W) House-surgeon (W)
	Ospringe *	One mile from Faversham. Many children suffered from ague in 1859	1859	Whitley
	Woolwich Purfleet Plumstead	Many cases of ague were treated among the out-patients of King's College Hospital, London	1845—1860	Lionel Beale ¹
	N. Aylesford		1850—1858	Peacock
	Hoo and Island of Grain	Gives mortality tables (see Text) (a) Cases of ague occurred among the navvies working in the marshy neighbourhood of Grain. In the Hoo Union, 347 (b) Cases occurred between 1852—1863	1864	Wright (surgeon) (W)
	Gravesend	(a) The dispensary records show 678 cases of intermittent and remittent fever between 1856—1862, including 371 in 1859 (b) Ague greatly on the decrease about this year	1852—1863 1856—1862	Whitley Whitley
	Wittersham *	Mr Robert A. W. Stevenson, chemist at Wittersham during 1867—1875, said there were hundreds of cases in the places named, and in others in Sussex (see below). He used to supply quinine to the people in the neighbourhood, and remembers when there were "7—8 patients of a Sunday morning." When he returned there in 1882 ague was very rare	1864	Armstrong (surgeon) (W) ²
	Stone *		1867—1875	
	Ebony			
	Tenterden			
	Appledore	Had seen much regular ague when he began to practise about 1833	1833	Adamson (surgeon) (W)
	Brookland *			
	Kennardington			
	Woodchurch			
	New Romney			
Sussex	Arundel	See note above under Bluntisham		
	Rye	Saw much ague (tertian) when he began to practise in 1824. There was a considerable increase in 1859	1824—1859	Deck
	Bexhill	Not much ague when he came to the neighbourhood (1844), and very little in 1864. The cases he saw were mostly on high ground at the edge of Pevensey Marsh	1844—1864	Wallis (surgeon) (W)
	Lewes	Very common	1826—1827	Sanby (farmer) (W)
	Glynde	St C. himself and several members of his family stated that they had suffered much from ague, but less of late years	ante 1864	Rev. St Croix (W)
	Beddingham	Intermittent disease very prevalent and in surrounding country	1864	Holter (surgeon) (W)
	Piddinghoe near Newhaven	Whitley was informed that a severe form of ague, not uncommonly quartan, formerly prevailed	ante 1864	Whitley
	Peasmarsh	See note to Wittersham in Kent above	1867—1875	
	Snargate *			
	Iden *			
	Playden			

¹ Letter to L. C. dated 20th Oct. 1900.² Stated by Mr Stevenson to G. H. F. N. 1900.

42 *The Geographical Distribution of Anopheles*

County	Place	Observation	Date	Authority
Hampshire	Lymington	In the early years of his practice, 1829—1864, had seen at one time a considerable amount of well-marked tertian	1829—1864	Adams (surgeon) (W)
	Christchurch	Much ague in 1827—1830 in the lowlands of the Avon and Stour	1827—1830	Welch (surgeon) (W)
	Lyndhurst	Ague lingered on somewhat longer than at Christchurch	1831	„
Somerset	Cannington	Ague endemic 1823—1828, the land being badly drained. This succeeded by 10—12 years, when only about 1·5 % of dispensary cases were malarious. In 1858 increased to 19 %, 94 cases occurring in a population of 800	1823—1858	A. Haviland ¹
	Chedzoy	Quartan ague occurred about Chedzoy	1864	Hurman (surgeon) (W)
	Bridgewater	The records of Bridgewater Infirmary show no case of ague amongst the in-patients for 1854—1855, three in 1858, two in 1861	1858, 1861	Whitley
	Huntspill	Told Whitley that this was always an ague locality. He had about 100 cases of ague, chiefly quotidian. He believes it had rather increased during his experience	1826—1864	Poole (W)
Cornwall		Lands End district hilly, dry and almost devoid of marsh-land and stagnant water. About 1796 many cases in this district, after which progressively diminished		
	Penzance	Only three cases reported in Penzance dispensary in period of 17 years amongst 8800 patients. Only one case during 11 years of Forbes' residence there, subsequently not a single case. Oldest practitioner had not seen a single case at Penzance during 50 years, nor in Cornwall for 12—20 years. Penzance dispensary register for 1810—13 gives two cases, 1819—22 gives one case, and none during 1823—33. Considerable decrease due to drainage, better houses, but admits something else is wanting for the explanation of the change there ³	1796—1822	Forbes ²

¹ Alfred Haviland, "Ague Epidemic at Cannington." *Journ. of Publ. Health and Sanit. Rev.* Vol. iv. 1858, p. 266.

² Forbes, J. (1836), "Medical Topography of Lands End and the Hundred of Penrith." *Trans. of Prov. Med. and Surg. Assoc.* Vol. iv.

³ The wanting element is possibly to be found in the decrease of ague in its marshy home and to the consequent cessation of foreign cases, thus rendering it impossible for the few local mosquitoes to become infected.

It having been incontestably proved in Italy, Holland, England and the United States that at least two species of *Anopheles* occurring in Great Britain are capable under certain conditions of transmitting malarial infection to man, we on the strength of our investigations have reached the following

Conclusions.

1. The disappearance of ague from Great Britain does not depend upon the extinction of mosquitoes capable of harbouring the parasites of malaria.

2. Three species of *Anopheles* (*A. maculipennis*, *A. bifurcatus*, *A. nigripes*) are to be found in Great Britain in all districts which were formerly malarious, but also in places concerning which there is no record of the former prevalence of ague.

3. The *Anopheles* to-day are most numerous in low-lying land containing many ditches, ponds and slowly flowing water, suitable for their habitat, and corresponding to the districts where ague was formerly prevalent.

4. Since the disappearance of ague does not depend upon the extinction of *Anopheles* it is probably due to several causes operating together:

(a) A reduction in the number of these insects consequent upon drainage of the land, this being in accord with all the older authors who attributed the disappearance of ague largely to this cause.

(b) Reduction of the population in infected districts as the result of emigration about the time when ague disappeared from England. This would naturally reduce the number of infected individuals and thus lessen the chance of the *Anopheles* becoming infected.

(c) It is possible that the use of quinine has reduced the chances of infecting the *Anopheles* through checking the development of the parasites in the blood of subjects affected with ague.

Of these, the first-mentioned cause seems to have been chiefly operative. The possibility is not yet excluded of there being another intermediary host besides man capable of harbouring the parasite, and, assuming that this were so, this host may have become extinct in the lowlands where it is known that the fauna and flora have altered.

5. The coincidence of the geographical distribution of ague and *Anopheles* as claimed by Grassi for Italy, and as probably holding good for other parts of the world, is hereby disproved for England, and

consequently the generalizations are proved to be premature whereby he excludes other blood-sucking insects from being possible hosts of malarial parasites on the strength of this supposed geographical agreement.

6. Since the geographical distribution of *Anopheles* in England is wider than the former distribution of ague in this country, we are forced to conclude that it is not a matter of the geographical distribution of *Anopheles* as much as of their *numerical distribution*.

7. Our observations having proved the existence of *Anopheles* in non-malarious districts, we believe that they will explain the occasional occurrence of ague in out of the way places, without making it necessary to assume that malaria-bearing mosquitoes have been freshly imported, for given suitable conditions of temperature and the requisite number of *Anopheles*, a malarious subject coming from other parts might well infect the local insects, which in turn would spread the infection to healthy persons.

8. We would suggest to those engaged in the investigation of malaria in other countries to search as carefully for *Anopheles* in non-malarious as in malarious regions. More data as to the number of these insects in various localities are certainly required, though we are fully aware that numerical estimates permit of a considerable degree of error. Nevertheless they would always possess a relative value.

STUDIES IN RELATION TO MALARIA.

II.

THE STRUCTURE AND BIOLOGY OF ANOPHELES

(*Anopheles maculipennis*).

THE EGG AND LARVA.

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THE importance of malaria as a disease affecting vast numbers of the human race renders it essential that we should study most completely all that affects the etiology of the disease. Through the brilliant researches of Ross, Grassi, Bignami and Bastianelli, and others we know that several species of *Anopheles* serve as definitive hosts of human malarial parasites, and that when these insects are infected they are capable of communicating the parasite to man. As far as the evidence goes these insects appear to be the only means by which the disease is spread. The sexually mature parasites circulating in man's blood gain access to the insect when it feeds upon this fluid. After a period of 7 to 8 days, under suitable conditions of temperature, the parasites appear in the insect's saliva, and when this occurs the latter is capable of producing infection by the act of biting. The highly interesting literature on this subject has been considered elsewhere by one of us, as also by Lühe, and the reader is referred to these publications for particulars¹.

¹ See Bibliography at the end of the Paper.

Other parasites besides those of malaria have recently been found to undergo their development in species of *Anopheles*. In 1878 Manson first observed the development of *Filaria bancrofti* in a species of *Culex*, and this observation has been confirmed by others. Quite recently Low (July 1900) observed that the matured *Filariæ* issued from the proboscis of *Culex ciliaris*; and James (1 Sept. 1900), working at Travancore in India, has proved that this parasite develops in *Anopheles rossii* and another undetermined species of this genus, as also in *Culex microannulatus* and *Culex albopunctatus* Skuse. Finally Grassi and Noè (Nov. 1900) claim to have observed a similar development of a canine parasite, the *Filaria immitis*, in *Anopheles maculipennis*. The latter authors state that the geographical distribution of Filariasis in the dog corresponds with the geographical distribution of malaria, as also of *Anopheles* in Italy. Their observations made by injecting filariæ which had matured in these insects though insufficient in number, certainly indicate that Low's hypothesis with regard to the mode of infection is probably true. The literature on Filariasis up to the time immediately preceding Low's discovery will be found in a paper (1900) by Nuttall to which the reader is referred.¹

It seems desirable to briefly summarize the recent advances not mentioned in the publications of Nuttall already referred to. Ziemann (21 June, 1900) working in Cameroon found two undescribed species of *Anopheles* in dwellings occupied by whites and natives². Of the insects examined 30% harboured parasites resembling those of malaria in their stomach wall. He reports that he was able to infect these insects with crescentic and tertian parasites, whereas these parasites did not develop in a species of *Culex* nor in Sandflies, the latter being very numerous. Van der Scheer and van Berlekom (1900), who have studied an outbreak of malaria at Middleburg in Zeeland (Holland), found that most of the cases occurred in the outskirts of the town, especially to the west. They noted house-epidemics, persons in the immediate neighbourhood remaining unaffected. The type of fever was single or double tertian. There were 110 cases in 45 houses. They found *A. maculipennis* in houses, stables, and huts, the insects feeding on human and animal blood. In July and August these insects were not infrequently found in human habitations. One-fifth of the *Anopheles* examined were found to harbour malarial

¹ See Bibliography at the end of the Paper.

² It is most desirable that investigators should state with what *species* of *Anopheles*, etc. they have experimented.

parasites in the stomach wall. They caught *Anopheles* and fed them on tertian blood, after which they observed the development of the parasites inside the insects. Of 5 insects kept at 18 to 21, 5° C., 4 became infected. Another observation showed that 18 out of 22 insects similarly fed became infected. When the insects were fed with blood containing immature parasites, with the blood of persons who were being treated with quinine, or with the blood of persons who had recovered from malaria the result was negative. Their observations show that *Anopheles* may become infected at a lower temperature than Grassi has claimed to be necessary in Italy. The publication is illustrated by means of micro-photographs showing the development of the parasites, as also by a photograph giving a very characteristic picture of *A. maculipennis*.

Manson (Sept. 1900) obtained *Anopheles* (spec.?) from Rome through Bignami and Bastianelli, these investigators having infected them with benign tertian parasites. The insects arrived in London after a journey of about 48 hours. Dr Manson's son, Mr Thurburn Manson, allowed himself to be bitten by the infected insects. Owing to his having been bitten by three lots of insects the period of incubation was not clearly established. The result of the experiment was however perfectly conclusive for Mr Manson subsequently developed tertian malaria. It has further been reported by Rees (Oct. 1900) that Mr George Warren was similarly infected by *Anopheles* (spec.?) imported from Rome. He acquired tertian malaria (benign) after an incubation period of 14 days. The experiments reported by Manson and Rees and made upon healthy individuals in a country free from malaria should fully convince those who may have remained sceptical.

Of 42¹ well-identified species of *Anopheles* eight or more have been proved to serve as hosts of the malarial parasite². Experiments with these species have been made in India, Italy, Africa, the United States, Holland, and England. There are three species known in England: *Anopheles maculipennis*, *Anopheles bifurcatus*, and *Anopheles nigripes*³.

¹ We are indebted for information with regard to the number of known species to Mr F. V. Theobald, who is publishing a monograph on the genus *Anopheles* from material supplied to the British Museum from all parts of the world.

² *A. maculipennis*, *bifurcatus*, *pictus*, *pseudopictus*, *nigripes* (?), *rossii*, *funestus*, and a few as yet undetermined species.

³ *A. bifurcatus* and *A. nigripes* are regarded as varieties of one species by Ficalbi (1899) and Grassi (1900). We have the authority of two eminent British dipterists (Messrs F. V. Theobald, F.E.S. and G. H. Verrall, F.E.S.) for the statement that they are perfectly distinct species. We are indebted to Mr Theobald for his determination of the

Of the three, the first, as will be seen from perusal of the preceding paper¹, is by far the most common, as it is also the most common and wide-spread species upon the European continent. It was whilst experimenting with this species that Grassi, Biguami and Bastianelli made the first studies upon the complete life cycle of the human parasites. For this reason it seems eminently desirable to give a careful and detailed description of this species, accepting it as a type of the genus all the species of which probably play a similar part in the propagation of malaria. We hope in a subsequent paper to supplement what may be lacking in the present publication. Though parts of this study may appear somewhat technical, the importance of the subject seems to us to warrant all the detail. There is scarcely a greater problem presented to modern hygiene than the extermination of malaria which renders vast portions of the globe almost uninhabitable to the white man. For this reason we should hasten to know all that concerns the insects which convey it.

Historical Note.

The first reference to *Anopheles* which we have found in the literature is that of Joblot (1754) of Paris, who describes the larva as rare, under the heading: "Description d'un nouveau poisson" or "Chenille aquatique." He gives a fairly good figure of the larva considering the instruments he must have used. Fischer (1812—1813) figures "*Culex claviger*," the larvae being those of *Corethra*. Meinert mentions that Gericke refers briefly to the larva in his *Zur Metam. d. Dipt. Gatt. Dixa*. Brauer (1883) mistook the larva of *Anopheles* for that of *Dixa*, his figure being poor, whilst his enlarged figure of the head shows that he mistook the ventral for the dorsal aspect. Meinert (1886) gives fairly good figures of the larvae of *A. maculipennis* and *A. nigripes*, the figures showing the whole larva, as also various parts studied in detail. Descriptions have been published more recently by Ficalbi (1899) and Giles (1900). The various stages in the evolution of *A. maculipennis* (Syn. *A. claviger*, *A. quadrimaculatus*) have been described and figured by Grassi and by Howard

following synonymy of the three British species above named: *A. maculipennis* Hoff. = *A. bifurcatus* Meig. 1804, *A. claviger* Fabr. 1805, *A. quadrimaculatus* Say: *A. bifurcatus* Lin. 1758 = *A. claviger* Meig. 1804, *A. trifurcatus* Fabr. 1792, *A. villosus* Robineau Desvoidy 1827, *A. grisescens* Stephens 1828: *A. nigripes* Staeger 1839 = *A. plumbeus* Haliday. We have added some dates from Ficalbi's monograph.

¹ *Studies in Relation to Malaria*, Part I. p. 12.

(1900). It cannot be said that any of the descriptions hitherto published give a full and satisfactory account or entirely accurate figures showing the development of *Anopheles*. As to the representations which have appeared in medical literature they have been either incomplete or very inaccurate.

I. THE OVUM.

When first deposited the eggs are white, but they soon darken. Each ovum measures 0·7 to 1·0 mm. in length and is at its greatest breadth about 0·16 broad. The egg is boat-shaped and one end is slightly deeper and fuller than the other. The surface which, were the egg a boat, would be the upper is flattened but slightly convex. It is marked by minute reticulations (Fig. 2). The under surface of the boat is characterized by much larger and more regular reticulations, which divide the surface into fairly equal hexagonal areas. The rim (*a*) of the boat is thickened and very regularly ribbed. Along the centre of each side, extending over a space of rather more than one-third the total length, this rim is much thickened, the ribbing is more marked and the whole forms a very conspicuous and characteristic feature of the egg. This thickening recalls the rounded float which runs along the edge of a life-boat (Fig. 1). It serves the same purpose, being composed of air chambers and is used to keep the boat-shaped egg with its flat surface uppermost. Howard (1900, p. 35) refers to the membrane we are about to describe as the "clasping membrane," notes the reticulated surface exhibited by the eggs, as also the presence of 5—7 minute dark circular spots at the ends. His measurement of the egg is given as only 0·57 mm. As in other insects the egg doubtless varies in size.

The colour of the egg soon after it is laid is grayish black. If the eggs are subject to much attrition a delicate membrane splits off which gives the surface of the intact egg its reticulated appearance. Stripped of this membrane, which desquamates in irregular whitish fragments, the egg appears with a glistening black surface comparable to that of patent leather. One end of the egg is slightly blunter and more rounded than the other, and this contains the head end of the embryo. It is an interesting point that when the egg, as frequently happens, is drawn by capillary action a little way up from the water on to a leaf or some other half-submerged object the head or blunt end always points downwards, and thus should the hatching take place whilst the egg is in this position the larva emerges into the water, and not into the air.

We have observed that the eggs floating upon the water slightly indent the surface-film.

According to Grassi (1900, p. 66) the female deposits about a hundred eggs upon the water. Howard (p. 35) says the eggs number 40—100. According to Grassi the eggs of *A. maculipennis* lie in groups of 3—20, side by side like a bridge of boats, whilst those of *A. bifurcatus* arrange themselves in star-like patterns, their ends being in juxtaposition. He only found the eggs of *A. superpictus* once, the eggs being scattered. The eggs of *Anopheles* do not adhere to each other as do those of *Culex*, the result being that they are readily scattered by the wind. It has however been observed in our aquaria that if left undisturbed upon the surface they tend to collect together, as do other small bodies even of an inanimate nature which float upon water.

Our observations do not in all respects confirm those of Grassi as to *A. maculipennis*. In the still aquaria of a laboratory the eggs certainly lie as he describes, but in the open pools in Great Britain, that is to say in their natural state, they seem to be invariably scattered. Empty eggshells are often met with, and they too in natural water about Cambridge are always scattered.

The eggs are laid upon water suitable for the development of the larvae, that is usually water rich in vegetable matter such as Algae. Grassi states that he first found eggs on the 15th of February, 1899. In the spring *A. maculipennis* and *A. pseudopictus* lay their eggs in water about 2 feet deep, later when the weather grows warmer the eggs are laid in water but a few centimeters deep. On the other hand Grassi (4 Oct., 1899) says that *A. bifurcatus* lays eggs in cool weather by preference in shallow water, especially such as contains cress. Grassi only found larvae in the end of March on the Pontine Marshes, their number increasing as summer advanced, whilst they were still encountered in September and October. In November his servant only found one larva of *A. maculipennis* after two days' search in the Campagna.

On the second or third day after oviposition, this depending upon the temperature¹, the young larva leaves the egg and commences to swim in the water. The egg hatches by means of a circular split near the blunt end of the egg-capsule. This separates a cap-like anterior

¹ Howard reports that eggs laid on the 26th of April hatched out on the 30th. Some others laid on the 13—14th of May hatched out on the 16—17th. On an average the eggs hatched out three days after being laid. He does not note the temperature, which is certainly important.

piece from the rest of the shell. There is no visible ring where the cap breaks off, but the cap is usually more or less of the same size.

Ross (23 July, 1900)¹ made an observation which indicated that the eggs possessed a considerable degree of resistance to desiccation. Some eggs of *Anopheles* kept in a test-tube hatched on being placed in water after a period of about six months. In a recent publication he throws doubt upon the accuracy of this observation, stating that it requires confirmation. It certainly finds no support from what has been seen by recent observers. Some eggs which we placed on filter-paper and under glass for less than five days did not give rise to larvae when floated upon the water of an aquarium. Christophers and Stephens (Aug. 1900, p. 8) found no eggs in dried pools at Freetown although they were present as long as there were moist cracks in the hollow which had contained water. The same authors state elsewhere (6 July, 1900, p. 48) that they found *Anopheles* eggs would hatch after being dried for 24—48 hours on blotting-paper, but that no larvae issued when more than 48 hours had elapsed. Tests made with earth taken from 25 pools gave a negative result. Gray (1900) of St Lucia, W.I., took mud from a pool that had been dried three weeks and added water to it, but the result was negative. Some grass taken from the pool margin and placed in water gave a positive result, in that larvae of *Culex tueniatatus* appeared within 24 hours. From these observations we see that the matter requires to be further investigated. It is quite possible that the resistance of the eggs may vary according to the season at which they are laid, and that the eggs of *Culex* are more resistant than those of *Anopheles*. We have no evidence to the effect that eggs can withstand hibernation, everything indicating that they are carried through the winter solely within the female's body, and only attain maturity in early spring when the weather grows warmer.

II. THE LARVA.

The body of the larva is divided into three regions:—(1) the Head, (2) the Thorax, (3) the Abdomen. The segments of the thorax are much fused together, still traces of a division into three are not wanting. The abdomen may be divided into nine segments. The first seven of these exhibit but a slight and gradual change of form, the eighth is rendered conspicuous by carrying on its dorsal surface

¹ Personal communication.

the stigmatic apparatus, and the ninth or last is equally conspicuous (Figs. 3 and 4).

The Head of the Larva.

The head of the larva is much more rounded than is usually represented, in fact the diameter from above downwards is very little less than from side to side, except anteriorly, where the dorsal surface slopes downwards and forwards (Fig. 7). The head is covered with a very complete and clearly defined chitinous case. The posterior edge of this is sharply cut and almost circular in outline. It is very little smaller than the biggest cross section of the head, and is strengthened by a slightly thickened band which runs like a clerical collar round the posterior, free edge. The chitinous cover is brown, lighter where there are joints, *e.g.*, at the insertion of the antennae; and darker where the chitin is thickened, *e.g.*, at the bases of some of the appendages. In *Chironomus* the ventral border of the chitinous covering of the head is cut away in the form of a bay, thus allowing the head to be flexed on to the thorax, but in *Anopheles* the edge of the chitin of the head is even and in no place curved in. In the dorsal middle line however the thickened band is cut in two and there is a narrow gap or slit between the two cut edges (Fig. 8). From the anterior end of this slit two lines of pigment diverge, forming the posterior sides of a diamond-shaped area on the upper surface of the head; there is in some larvae also a median ventral slit.

The eyes are situated quite laterally, and they seem to be of two kinds, one is compact and more or less circular in outline, the other, which is only visible in the older larvae, is a cycle-shaped body compounded of isolated ommatidia. These lie above and a little in front of the rounded eye, and it would seem that they are beneath the transparent cuticle and are in fact the primordia of the adult eye. The level of the eyes is at about the juncture of the posterior third with the anterior two-thirds of the head. Between them on the arched upper surface the head bears a line of four symmetrically arranged branching¹ hairs, which are difficult to see (Fig. 4).

In front of the eye at about the same horizontal level is an eminence which carries the antennae, and this eminence is partly caused by a slight but deepening groove which runs in front of the eye and helps to mark

¹ We have used the term *branched* when the branches of the hair in question lie in more than one plane, *feathered* when they lie in one plane.

off a *central* area of the upper surface of the head from a *lateral* portion which is continuous with the sides. Between the two eminences a broadish band of pigment runs across the head on the dorsal surface, and this bears six symmetrically placed feathered hairs which project forward over the head (Figs. 4 and 7). These remarkable and characteristic hairs never seem to move, the tips of the central hairs reach forward in front to the anterior end of the head, over which they hang like a kind of "glory." It may be that they serve as a buffer to shield the head, but judging from the usual movements of the larva, which generally take place tail foremost, danger from this cause is little to be apprehended.

Anteriorly the head which has narrowed,—since the groove above mentioned has deepened and passed on to the side,—is cut off by a sharp edge and the most anterior end of the animal is formed by an area supported above and on both sides by the chitinous edges of the head but running into soft tissue on the ventral side in front of the mouth. This membrane is almost vertical, but slopes slightly backwards and downwards. At each corner of the dorsal chitinous end of the head is placed a conspicuous branched hair (Fig. 4), which exactly overhangs the brushes shortly to be described. In the middle line or rather close to it, two simple pointed hairs (Fig. 4) slightly frayed at their free ends project forward. These are very fine, and lying as they do with their ends near together often look like a single stout hair, closer examination usually shows that the tips are crossed and that there are really two hairs¹. Grassi (1900, Plate IV.) has drawn attention to the fact that these branched hairs at the corner and the two simple or slightly frayed hairs near the middle are of specific importance and has given figures showing the differences which exist in *A. claviger*, *A. pseudopictus*, *A. superpictus* and *A. bifurcatus*. There are other smaller hairs on the head but those mentioned are the most conspicuous.

The anterior end of the head carries on each side of it a very conspicuous bunch of stout, dark brown hairs (Figs. 7 and 8) which have some sort of a spiral arrangement and are slightly curled. The hairs are as closely aggregated as those of a shaving-brush. Between the bases of the two brushes is a smaller bunch of hairs, and ventrally there are two semicircles of hair, all above, in front of and converging on the mouth.

The anterior median area which carries the brushes is called by Meinert the 'clypeus.' In older larvae it is produced into a conical spine or process, very difficult to see, which underlies the two median

¹ For the sake of clearness these branched and frayed hairs have been omitted from the enlarged views of the head (Figs. 7 and 8).

hairs. This gives a kind of finish to the head but it does not seem to be present in the younger larvae. There is also a pair of chitinous bars in this area which will be considered more in detail when an account of the head muscles is given.

The muscles which move the bunches of hairs are two pairs, symmetrically placed. The internal pair have their origin from the dorsum of the head, the point being indicated by a symmetrical patch of pigment situated in the posterior angle of the diamond-shaped area on the head about the level of the eyes. The muscles arise almost in contact and run almost parallel, but very slightly converging to the middle anterior end of the head where they are inserted on the soft membrane, but between the bases of the brushes.

The external pair of muscles have their points of origin separated by a space about equal to half the diameter of the head. Their origin is also marked externally by a patch of pigment which lies just within the lateral angles of the aforementioned diamond-shaped area. These muscles are stout and converge towards the anterior end of the head where they are inserted into the area which bears the brushes, but external to the median or internal muscles. Their direction is inwards, forwards, and slightly downwards. Their point of origin is within and in front of the eyes.

A third pair of stout muscles have their point of origin near the posterior edge of the head-case and are more laterally placed. In fact they are external and posterior to the eye-complex whereas the relations of the point of pigment of the external brush muscles to the same organ are internal and anterior. These muscles run more obliquely downwards than do either of the above-mentioned pair. We have not been able to follow them to their insertion, but we have no doubt they are inserted into the maxillae and that their contraction causes the very vigorous movement of the organs whilst the animal is feeding.

In a future Paper we hope to give a full account of the muscles of the head, here we must content ourselves with the bare mention of the three largest.

The Appendages of the Head.

The only appendages borne by the larva of *Anopheles* are those of the head, though possibly the larger hairs of the thorax may correspond in position with the future legs.

The head appendages are paired and consist of (i) Antennae, (ii) Mandibles, and (iii) 1st Maxillae.

The antennae may be described as two jointed, though the first joint which is quite short seems immoveably fixed to the chitinous covering of the head. The second joint is elongated, moveable, and provided with a few spines. Its free end is truncated, and bears two large spines slightly curved. These can be separated one from another and brought together again. Between the bases of these spines a very minute spine has its origin and also a branched hair which is rather longer than the large spines (Figs. 7 and 8).

The mandibles consist of one joint forming the side of the mouth, which is floored by the maxillae (Figs. 7, 8 and 11). Each mandible is a stout piece, articulating by a broad base with the under, lateral part of the head. Its free, anterior border bears passing from without inwards the following structures. Most externally three, sometimes four (?) strong cycle-shaped hairs which in a position of rest often touch the posterior, outer angle of the brushes of hairs, and so help to form a kind of sieve to entangle any particles of food which may be approaching the mouth. These hairs are sometimes run through the brushes and serve to clean and arrange them. More internally the mandible is produced into a number five or six, stout, chewing teeth which judging from their colour must be strongly chitinized. These teeth working against those of the opposite mandible are the only crushing apparatus the larva possesses. More internally still is a bunch of hairs which line the mouth (*l.* Fig. 11).

The larva of *Anopheles* has a single pair of maxillae, the first. There is nothing which can be homologized with the second pair. Each maxilla is a somewhat flattened, quadrilateral strongly chitinized piece, the inner edge of which approximates to its fellow of the other side but does not touch it. In the angle between them lies a conical, toothed piece—the “under lip” of Meinert (*q.* Fig. 8). The maxillae take by far the largest share in flooring-in the space in which the food-particles accumulate. The upper surface of each is covered with fine hairs like a carding brush, these are arranged in two areas separated from one another by a space devoid of hairs, which runs from the anterior, inner corner obliquely backward. The hairs of the inner area point outwards and forwards, those of the outer inwards and forwards (Fig. 12). At the anterior edge some of these hairs project and are curved, and these are especially well marked towards the outer side. From this description and from Figure 12 it will be seen that the inner surface and the anterior edge of the maxilla act as a most efficient brushing or combing instrument which cards through the hairs of the brushes and

clears them of any particles entangled in them and combs them out. The maxilla is united to the head by a wide, spacious joint.

The maxilla bears on its outer edge a palp which lies in a plane slightly dorsal to the maxilla and helps to form the sides of the above-mentioned space in which the particles of food are collected together. The palp consists of a single joint which bears at its end three spines. Between the spines in a manner indicated in Figure 12 a thin lamella of very definite shape but of a soft membranous nature projects. The single joint bears many minute hairs and a cluster of longer ones towards the end, close to the spines.

Method of Feeding of the Larva.

On the extreme anterior end of the head are the two closely packed bundles of slightly curved fine, hair-like setae, which we have throughout this Paper called the brushes. Other observers have called them the rotatory, whirling and vortex organs, a name which suggests that the individual hairs move in succession and create a whirlpool. This is however not the case. All the hairs move together and both organs move as a rule simultaneously and in the following way. The hairs are arranged in a sort of cupped, fan-like shape, something like a very strong, overhanging, arched moustache. Their point of origin is attached to the extreme anterior edge of the head, and this portion of the head, the "clypeus" of Meinert, is hinged on to the rest of the head; the whole organs can be bent on this hinge, and when bent the two bunches of hair also tend to come together towards the middle line. The two brushes however can move independently, and at times one is seen bent under whilst the other remains erect.

Whilst feeding at the surface, which seems to be the chief occupation of the larva, the head is reversed so that the ventral surface lies uppermost, the body retaining its normal position. This peculiar rotation of the head does not take place in the larva when it feeds at the bottom. Almost immediately upon returning to the surface it suddenly turns its head on its neck as an axis, through an angle of 180° . This is done suddenly and with such precision that one almost expects to hear a click. The mouth-parts now begin to vibrate backward and forward and the brushes are bent rapidly downwards, backwards, and inwards. When at rest, the outer end of the curved moustache is almost touched by the free end of the maxillary palp (Fig. 8). This organ helps thus to form the sides of a space at the bottom or posterior

end of which the mouth lies. The walls of this chamber are completed by the mandibles, the curved hairs of which, whose function is considered above, are frequently projected so as to just cover the tips of the outermost hairs of each brush. The floor of the above-mentioned space is mainly formed by the two flattened maxillae, which curve under like the ribs of a boat and almost meet in the middle line. Their inner borders are separated by the conical process with two or three teeth on each side, the so-called "under lip" of Meinert. Together with the maxillae it floors the space into which the brushes are bent back, and which is roofed by the under surface of the head.

When the larva is feeding the brushes are suddenly bent back into this space, the mandibles and maxillae moving forwards to meet them and at the same time opening out, they are then as suddenly released and fly back to their original position. This movement of sudden bending and swift relaxation is repeated with great rapidity, often some 180 times a minute, producing a current sweeping in convergent curves towards the above-mentioned cavity. The water filters out on each side, but any particle of food is retained by the complex of fine hairs which are borne by the mouth appendages.

From time to time the mandibles are approximated and the stiff curved hairs of their upper edge are run through the brushes like fingers through the beard, and thus the mandibular curved hairs help to arrange the hairs of the brushes and keep them in their order. They may also assist in removing any particles of food that may be entangled in the brush. That this is of importance is shown by the fact that at intervals, generally at the end of a certain number of contractions, the brushes disappear far into the mouth and are then slowly withdrawn, passing through the fine carding bristles on the inner face and anterior edge of the maxillae. In this way any particle of food which may have become entangled on the brushes is carefully separated and remains on the mouth side of the maxillae. The brushes are frequently swallowed again and again and withdrawn in little jerks, so that the fine teeth-like hairs which act as a carding instrument have every opportunity to comb out any particles entangled in them. It is a most fascinating operation to watch.

From the position of the larva when feeding, the head with its brushes lies close below the surface-film, the currents set in motion by the action of the brushes extend to a distance equal to twice or thrice the length of the larva or even further. The currents seem to be in a plane just below the surface-film and to affect the organisms and

organic *debris*, which being lighter than water, float up from the bottom of the pond or puddle and lie under the surface-film. In fact the larva sweeps the *lower* surface of the surface-film of the water, just as a ceiling might be brushed to remove the flies and spiders which may have settled there.

Food of Larva.

The food of the larva seems to consist in the main of spores of fresh-water algae, particles of *Spirogyra*, diatoms, and any other minute organisms which do not penetrate the surface-film. The larva may be seen to browse about the decaying leaves of *Lemma*. They do not feed for any length of time beneath the surface, and when they do so the head is held in the normal position and moved about somewhat like that of a caterpillar, the insect browsing about over the surfaces of sunk particles overgrown with algae, etc. We can confirm the observation of Grassi (1900, p. 58) that larvae are best reared when there are few in the aquaria. We have always taken the precaution however to remove any other animals that were inimical. We have on several occasions observed larvae devouring their dead fellows. Gray (1900, p. 583) of St Lucia, W. I., also finds that *Anopheles*' larvae there are not altogether vegetable feeders, as some would appear to believe, and has likewise seen them feed "on the dead bodies of drowned mosquitoes." Grassi (1900, p. 58) thinks that a small amount of *Lemma* in aquaria favours the growth of larvae, though they develop best in the presence of confervoid algae. He found the intestine to contain protozoa, unicellular algæ and organic detritus. Howard (1900, p. 39) observed that the dark central axis of the larvae (*A. maculipennis*) was due to the nature of the food contained in the intestine, and that when the larvae were fed on algae their bodies turned green. Finally Christophers and Stephens (Aug. 1900, p. 3) found *Anopheles*' larvae in Africa feeding chiefly on a unicellular organism (*Protococcus*?).

At times some structure larger than the larva can swallow is involved in the current set up by the brushes. A short, sharp struggle ensues and after an effort or two the morsel is rejected. The particles that are swallowed accumulate for a certain time, until a mouthful of some size is attained and this then suddenly passes into the oesophagus.

As is well known the larva of the gnat, *Culex*, hangs down into the water attached to the surface-film only by its respiratory siphon. It

sweeps the water for food some two or three millimeters below the surface. It is devoid of the palmate hairs which enable the *Anopheles* larva to float with the whole length of its body close to the surface-film. The habit of feeding upon matter adhering to the surface-film has only been observed by us in the larvae of two insect genera; *Anopheles* and *Dixa*¹. The fact that certain fresh-water Snails and many *Turbellaria* browse over the same area is further evidence that as a feeding ground it is by no means unworthy of notice.

The Thorax.

In youngest larvae the head is decidedly broader and deeper than the following segments, in older larvae (Fig. 3) the segments which follow the head have surpassed it in the area of their cross sections, whilst in the fully grown larvae (Fig. 4) about to pupate the segments which succeed the head are at least twice its diameter.

¹ The larva of *Dixa*, which possesses a resemblance to *Anopheles*, and has often been mistaken for it, also floats beneath the surface-film. We have caught about 14 *Dixa* larvae this summer in the same places where *Anopheles* were captured. *Dixa* larvae may be distinguished at a glance from those of *Anopheles* by the fact that the "respiratory-cup," as Miall (1895, p. 157) styles it, is much larger and that only this organ and the vibratile mouth-parts touch the surface. The larva has no palmate hairs such as enable *Anopheles* to float flat, and the body of the *Dixa* larva is usually submerged with its dorsal surface concavely arched. The area of the respiratory-cup of *Dixa* is considerably increased by numerous simple marginal setae arranged like pine-needles on a stem, and it is owing to the greater size of this organ that it alone suffices to maintain the larva near the surface. Whilst the thorax is large in *Anopheles*, all the segments in *Dixa* are of fairly uniform size. Whereas *Anopheles swims tail first and downward* when disturbed, *Dixa swims head first*, and usually maintains itself upon the surface. In swimming only the anterior four segments lash *laterally*, the remainder of the body remaining rigid. Examined microscopically *Dixa* is seen to possess four *prolegs* ending in hooklets as in caterpillars, these prolegs issue anteriorly and from the ventral surfaces of the 4th and 5th segments. Whilst *Anopheles* in feeding on the surface *rotates* its head so that it turns bottom side up, *Dixa bends its head backward* upon its neck so that it forms a right angle or less with the body. The result is that whilst *Anopheles* attracts particles from in front towards the head, *Dixa* attracts them from behind. By using its prolegs *Dixa* creeps up the side of a vessel or stem, the anterior and posterior portions of the body remaining pendulous. Already Réaumur (1714, *Mém. de l'Acad. Roy. de Paris*) compared this position to that of a *siphon* and observed the larva advance or retreat with the water at the edge of the vessel when the latter was tilted to and fro. Figures of this larva will be found in Meinert (1886) and Miall (1895). As the latter points out the *Dixa* larva does not break the surface-film in creeping up the side of a vessel for it drags up a part of the film with it. He has however observed these larvae occasionally wander out of a vessel and perish from drying up. When the larva sinks a bubble of air is often carried down in the respiratory-cup, and the larva when below swims somewhat like a worm. From this short description it will be seen that there should be no difficulty in distinguishing the larvae of *Dixa* and *Anopheles* even with the naked eye.

Throughout the Diptera in the imago "the thorax is remarkable from the absence of distinct separation into the three divisions which may usually be so easily distinguished in Insects¹," and this peculiarity is exhibited in the larval *Anopheles*, as far at any rate as the external features go.

The thoracic region in the older larvae is broader than the head and broader than the succeeding segments, which taper very slightly to the tail.

On each side of the middle dorsal line of the anterior rim of the thorax is a line of three feathered hairs, increasing in their size from within outwards and spread forward overhanging the head. Overhanging the base of these is a curious, flattened, notched process, only to be seen when under a very powerful light (Figs. 4 and 6). On the ventro-lateral border on the same edge emerge on each side a pair of large feathered hairs, sometimes double from the base, these resemble the dorsal hairs and also those which are now to be described.

A very little way behind this row of hairs come on each side of the prothorax two hairs usually feathered; these are distinctly lateral in position and are almost always directed forward, even when the first and third rows are standing out at right angles to the body. This second row of four hairs is not so conspicuous as the others and do not seem to conform to the same series, but for this they might be looked upon as the bristles of the mesothorax.

At a greater distance than separates row one from row two comes row three, which is in fact near the posterior edge of the thorax. This consists of a row of four feathered hairs projecting as a rule forward, though often outward. Each row of four hairs is lateral, and not unfrequently one of the four is broken off. There are numerous small, usually simple hairs, for the most part symmetrically arranged on the thorax besides these specially mentioned.

The Abdomen.

The first two segments of the abdomen, which consists of nine segments, each bear at their lateral, posterior angle a pair of similar bristles. Where they arise the surface of the segment is projected almost like a parapodium. The third segment, at any rate in younger larvae, carries but one of these feathered hairs. Each is feathered, *i.e.* branched in one plane only, and this plane is at right angles to the

¹ D. Sharp. *Cambridge Natural History*, Vol. vi. p. 445.

long axis of the body. They protrude out from the body, arching slightly forward, for a distance on each side equal to at least double the width of the body in the younger larvae. They undoubtedly act as balancers, like the sculls of a rowing skiff, but they do not seem to exercise independent movement.

Near to the base of these large lateral hairs, and on a line which slopes inwards and slightly forwards, are on each side of the dorsal surface four small hairs. The most external of these is a branched hair with a very short stalk, and the branches of which are simple, fine, rounded hairs projecting in a bunch. The second hair passing inwards is a single, straight, unbranched hair of some length, about as long as the body is broad, the third and fourth hairs are branched like the first but are much smaller, and the fourth is smaller than the third (Fig. 13).

Nearer to the middle line than the innermost of the four hairs just described, but not in the same line, being a little posterior, but distinctly on the upper surface of the animal is a small conically branched hair. On the 1st and 2nd segment behind the thorax this hair is small and inconspicuous, but on the 3rd, 4th, 5th, 6th, and 7th segments they are highly modified and play a very important part in the life of the larva. We call these five pairs of specialized hairs *palmate* hairs. The significance of these hairs seems to have been hitherto entirely overlooked.

Each of the hairs has a little but very distinct stalk, like the handle of the framework of an umbrella (Fig. 5). At its free end this stalk bears a conical bundle of fine hairs placed like the ribs of the umbrella if one imagines it turned slightly inside out, about one-third of the ribs missing and the remainder somewhat flattened and spindle-shaped in outline. The whole forms a most delicate little cup, and it is by means of these five pairs of palmate hairs which cling on to the surface-film that the larva maintains its position close under the surface of the water. This cone is not quite complete, a few of the inner hairs wanting, in fact the circle wants a segment of about 80 % on its inner edge. The palmate hairs may at times be seen to enclose air-bubbles when the larva is submerged.

The large lateral feathered hairs which form a series along the side of the thorax, and the first two post-thoracic segments, become as is mentioned above single on the third post-thoracic segment and on the fourth is represented by a single hair which has two branches only, and on the fifth by a single unbranched hair, on the sixth and seventh the corresponding hair is small and insignificant whilst the branched hairs

internal to it on the dorsal surface become larger and more conspicuous as we pass backward (Fig. 4).

The hairs on the ventral surface are either simple, unbranched, or they have a short stalk which ends in two or three, sometimes more, straight simple hairs diverging at equal angles. One of the later lies about on a level with the large hair or its representative, another lies a little internal and anterior to this. A third hair, and this time a simple one, lies still more internal, the bases of these three making a roughly speaking equilateral triangle. In front of the third single hair and in a line with it lie two very small hairs (Fig. 14).

This is roughly speaking the arrangement on the fourth abdominal segment, on the fifth one of the hairs which appears to correspond with the forked hairs above the chief hair becomes feathered and lies backward, and on the sixth and seventh two, one above and one below, become similarly feathered and lie back, overlapping the following segments just as the feathered hairs on the thorax and first and second abdominal segments lie forward, though in size the two are not at all comparable. The exact arrangement and position of these hairs appears to differ after each moult.

The eighth abdominal segment is modified in connection with the opening of the respiratory apparatus, but this chiefly affects the dorsal surface, the hairs on the side and under surface do not suffer much change although the large feathered hairs projecting backward are absent or modified. The hairs which seem in relation with the stigmatic apparatus are described with that organ.

The ninth segment at the posterior end of which opens the anus is modified in shape, being no longer rather flattened and squarish in cross section but round, and the whole segment is cylindrical. It bears some very remarkable hairs, but with the exception of these which will shortly be described and two single hairs which proceed from the side about half way along the cylinder, the hairs which are so characteristic of the other segments are wanting. The surface of this last segment is beset with very minute pointed bristles, all pointed backwards and giving the portion of the skin so characterized a shagreen-like appearance (shown in Fig. 3).

The posterior end of the body is cut off sharp and presents an oblique, plane, round surface (Fig. 9). Near the centre of the round disc the anus opens, a little ventral to the exact centre. Surrounding it, and placed dorso-laterally and ventro-laterally on each side, quite symmetrically are four soft, white, anal papillae, which when fully extended may

attain a length almost two-thirds as long as the ninth abdominal segment. The anal papillae are well supplied with tracheae and are clear, transparent structures with considerable powers of retraction. The posterior end of the rectum is liable to a prolapsus and often extends some distance out of the anus, this is, we fancy, the result of pressure and does not occur in nature except at the moment of evacuation.

On the dorsal side of the posterior rim of the ninth abdominal segment are four very prominent and remarkable hairs: two median and two lateral (Fig. 9). They hang back over the anus in a very characteristic and graceful manner. The two right and the two left hairs arise in close proximity, but the median right and the median left, which also arise close to one another and to the median line, immediately after their origin come to lie in contact, and thus it requires careful observation to see that there are in reality two hairs and not one. The hair is of the feathered variety, the branches being in the horizontal vertical plane, and the first four or five are on the dorsal side only. Nearer the tip, however, we find branches on each side of the central axis. The two feathers hang back over the anus in the median vertical plane, but are arched up dorsally. The lateral right and left hairs are not so much feathered as the central, indeed they are strictly speaking not feathered at all but forked, the four or five branches into which they split all arising at about the same level. These two hairs project backwards and slightly upwards and outwards, and are symmetrically arranged. The four form a very beautiful structure.

Ventrally the ninth abdominal segment bears a wonderful fan-shaped arrangement of hairs springing from two skeletal pieces of singular structure. The basal apparatus is paired, and each half resembles a quarter of a solid oval. The convex surface is externally, one of the straight sides looks upwards and the other faces the corresponding surface of its fellow. Inserted along the convex surface of each is a uniform row of nine feathered hairs, which have their origin in a very distinct circular articulation. The hairs of one side are closely applied to the hairs of the other, pair after pair, so that seen sideways the apparatus looks as though it consisted of but one row of hairs (Fig. 9). The longest hairs are the third, fourth, fifth, and sixth, at each end they diminish in size so that they together form a very beautiful fan-like structure. The feathering is more or less in one plane, and the hairs end in long, sweeping, very pointed free ends. At the anterior end of the apparatus medially is a single small hair.

When the larva is at rest hanging on to the surface-film this ventral fan hangs down into the water and presents a graceful appearance, but when the larva seeks the bottom of the vessel and comes to lie on its ventral surface the fan is rather in the way and is bent to one side. This elaborate apparatus may serve as an accessory organ of locomotion, though its delicate structure certainly suggests some sensory (tactile ?) function.

The base of the apparatus is further supported by two fine bars of chitin which run forward in the skin. In fact, all the hairs all over the body except on the head, which is uniformly chitinized, are supported by a small, oval plate of chitin with which they articulate. These plates are let into the skin, in fact are parts of the skin chitinized.

The description of the hairs given above applies, roughly speaking, to all the larval stages, it is mainly taken from a medium sized larva. In quite young forms the hairs are smaller and a few may be absent. At any time during the larval existence the hairs may be injured or even broken off. In the older larvae this seems to be especially the case.

Respiratory Openings.

On the eighth abdominal segment are situated the external openings of the respiratory system supported by a somewhat complex skeleton. In *Culex*, as is well-known, the larva in this region of the body gives off a long respiratory tube directed dorsalwards. This is much larger and longer than the last segment of the body, and its presence gives the larva the appearance of a Y with unequal limbs. In *Anopheles* there is no such tube but the two large tracheae open to the surface by two stigmata which are surrounded and supported by a complex apparatus.

The easiest way to understand the apparatus (Figs. 3, 4 and 15) is perhaps to compare the larva's body to a round stick of soft wood; at one end of this, corresponding with the eighth abdominal segment, we must imagine that a chip has been cut, but remains still attached to the stick though standing out from it. The posterior surface of the chip or the lobe which represents it in the *Anopheles* is held off from the body by a chitinous ring which forms some two-thirds of a circle; at the sides where this ring is most prominent it forms a curved flattened plate with prominent teeth projecting a little outwards and backwards (Fig. 15). Of these teeth there are some seven large, stout and dark, whilst between and within their bases is a row of smaller less chitinized teeth. These all overhang and guard the space between the chip or

the lobe and the body, and hold up and keep the lobe standing out from the surface of the body. The two toothed lateral arches are joined together by a thin chitinous bar which runs through the lobe and is continued along the base of the arches to their ventral end, where it splits into a small fork. The recess which is overhung by the lobe and protected laterally by these toothed plates is not the respiratory depression. That is on the dorsal anterior surface of the lobe. Here in the middle is a small squarish space bounded laterally by two thin incurved more or less chitinous plates which are rolled in towards one another like a piece of paper bent into half a cylinder. In front of these and between them and the fan-shaped piece, to be mentioned in a moment, are two minute triangular flaps. The anterior boundary of the square area is formed by an outstanding chitinous plate, fan-shaped and stalked, and the stalk runs downward to the middle line of a curiously chequered plate which forms the floor of the area (Fig. 15). Posteriorly the lateral curved-in plates bend in towards one another and unite in a median posterior plate.

The stigmata lie in the anterior lateral corners of this area, closely tucked into the corner and overshadowed by the median anterior fan-shaped plate. They are circular in outline with a well-marked thick rim. When breathing the animal lies hanging on to the surface-film by means of its five pair of palmate hairs described above, the edges of the respiratory organ pierce the film and the air is in contact with the squarish area and can enter and leave the trachea. When spread out, the side pieces are more or less unrolled, the triangular flaps are laid back, and the fan-shaped flap bends forward so that its free edge lies anteriorly. The stigmata are most fully exposed to the air. If anything tends to frighten the larva the side pieces and the triangular flaps are curved inwards, the fan-shaped piece folds suddenly back, the connection with the surface-film is broken and the animal darts suddenly below, and frequently carries with it a drop of air attached to the rim of the respiratory recess. Very often the larvae cease lying parallel to the surface, remaining attached only by the edges of this recess, and the anterior end of the body hangs down into the water. At times we have noticed the larva twist itself into a loop and begin cleaning and clearing this complex respiratory apparatus with its mouth organs. We have also seen it carefully clean the posterior bristles which sometimes are covered with *débris*.

When breathing freely the fan-shaped plate is bent forward so that its posterior face looks upwards, but often it is bent backwards with its

posterior face downwards. In the former position one can look straight into the stigmata, but in the later the stigmata are partially or wholly covered in. When the larvae leave the surface-film they sink by their own weight, but more generally hasten their retreat by actively swimming downward. This they do by rapidly bending the body first one side and then the other, forming a series of SS, very eel-like. Similarly when leaving the bottom to resume their position beneath the surface-film they swim upwards rather obliquely, until the tail touches the film, when they are suddenly arrested and stop. Very often they shift their position upon the surface by a single sharp side stroke of the tail. They invariably move tail forwards, and the hairs of that part of the body undoubtedly act as buffers. When on the surface they are usually feeding but by no means always, when at the bottom they as a rule lie motionless as if feigning dead. In a glass beaker they are apt to lie with their tails attached to the small concave film which capillary attraction draws up the inside of the glass. In this case the bodies lie radially, the heads pointing towards the centre of the beaker. If kept below the surface, say by a watch-glass, they frequently breathe by attaching the respiratory apparatus to an air-bubble.

At the anterior end of each of the post-thoracic segments on the median dorsal line is a small brown chitinous tergum, this is inconspicuous in the younger stages, and even in the largest larvae it is small. The plates are elongated in a transverse direction, they overlie the heart and are just about as broad as that organ. In the larger larvae they are perhaps about one-fourth or one-third the total width of the segment which bears them, at its narrowest point, which is in fact at the anterior end of the segment just where these terga lie (Fig. 4). They and the other chitinous thickenings, such as the bases of the hairs, are best seen in the cast, larval skins.

After leaving the surface when frightened or otherwise the larvae remain usually below for $\frac{1}{2}$ to 3 minutes, but we have seen them remain as long as 14 and 25 minutes in this situation, alternately feeding and resting at the bottom of the aquarium.

Colour of the Larva.

The general colour of the younger larvae as seen by the naked eye is black, seen with a lens the head appears of a symmetrically mottled brown, darker where the chitin is thick, lighter where it is thin or

newly formed. Frequently a light annulation is visible about the prothorax and the tail also is light. From behind the head until about the last two abdominal segments the body appears of a plumbeous black hue. At all times it is the centre of the body that one sees, the sides are so transparent as to be hardly visible, and even when the thorax is much bigger and broader than the head this is often not obvious to the naked eye because the side to whose growth the increased width is due is so transparent.

The older larvae retain their mottled brown colour on their head, but in them the body is lighter than in the young, very often green. This may be due to the food. Down the centre of the back runs a whitish streak, enlarging in each segment, which seems to be caused by some cells along the pericardium. This streak is broken only by the black-brown terga, and each side of these chitinous pieces the white is densest. The green colour is not only in the intestine but at times permeates lightly the tissues in the side of the body, which in the younger larvae are colourless. In the older larvae the five pairs of palmate hairs stand up as black spots on each side of the 3rd, 4th, 5th, 6th and 7th segments, and are well seen in larvae 7 mm. long, in which the thorax = 1.5 mm. broad. After moulting the larvae—at least some of them—are of a lightish lavender colour, very uniform, but they soon darken.

Notes upon the behaviour of Larvae.

The larvae lie with the long axis of their bodies parallel to the surface of the water. At times, though exceptionally, only the respiratory apparatus is applied to the surface-film, the axis of the body forming a slight angle with the plane of the film. Viewed from the side the respiratory apparatus as also the palmate hairs upon the dorsal surface of the abdominal segments are seen to indent the surface-film. The vibratile mouth-parts also disturb the continuity of the surface-film. The palmate hairs just referred to produce a series of minute bilateral indentations in the film, making it appear on superficial observation as if the dorsal surface of the larva actually protruded above the surface. It might be added here that the eggs, as also the respiratory trumpets of the pupae, likewise indent the surface-film. When the wind passes over a pool or ditch containing *Anopheles*' eggs, larvae and pupae, these will gradually be driven towards the opposite side from which the wind is blowing, though a certain number may

remain in places sheltered by an overhanging bank. This is due to the impact of the wind against the indented surface of the film.

The head is supported upon a long, thin and muscular neck. The neck is not visible in living larvae, the head seeming to project directly from the thorax. The length of the neck is only fully appreciated in dead larvae, where the parts are relaxed and possibly distended owing to the gases of decomposition accumulating within the body of the larva.

When disturbed the larvae wriggle rapidly tail first to the bottom of the tank, where they lie motionless on the ventral, lateral, or even dorsal surface, the body remaining extended. It is probable that they elude their enemies to some extent by this behaviour. After a few moments, if left undisturbed, they again wriggle tail first to the surface, and soon resume feeding, which in connection with their normally rapid growth is their chief occupation. Whilst feeding, provided they do not change their position through wriggling, the larvae lie stationary beneath the film, only the mouth parts moving. *Culex* larvae on the other hand, which only adhere to the surface by means of the respiratory apparatus, which when open offers but a small point of attachment to the surface-film, are seen to be continually carried forward through the motion of their mouth-parts alone.

The essential differences between the larvae of *Anopheles* and those of *Culex* have been dwelt upon by a number of recent writers. The *Culex* larva has a very large, broad head, which owing to its weight causes the insect to float head down and almost vertically in the water. It possesses a long respiratory tube containing the two terminally enlarged tracheae, which serve as floats. The respiratory tube ends in five leaf-shaped flaps, which are opened like the fingers of the hand when applied to the surface, and approximated to each other when the insect leaves the surface. Whereas *Anopheles* is mainly a surface feeder, *Culex* often feeds at the bottom, browsing about in its normal position, tail uppermost. The larvae of *Culex* are more readily frightened than those of *Anopheles*, and return more slowly to the surface than do those of *Anopheles*, a fact which renders it easy to separate the two genera by the use of a pipette, when found together in a given sample of water. If the larvae of either species cease to move whilst suspended beneath the surface of the water, they are seen to gradually sink to the bottom by virtue of their weight, *Culex* invariably head-first.

The larvae are not supplied with limbs, locomotion being effected by rapid wriggling motions throughout their length, the direction

taken being tail-foremost, but otherwise erratic. Possibly the long hairs upon the lateral surfaces, and certainly the long hairs at the terminal segment, act as accessory organs of locomotion, though not of themselves necessarily moveable.

Observations upon the growth of Larvae.

The following observations were made in tanks in the laboratory, the tanks being cylindrical glass vessels about 25 cm. across and containing water about 6 to 8 cm. deep. The tanks were placed near a window where they were exposed to the sunlight during several hours of the day. Fresh *Spirogyra* and fresh water were added from time to time. It was found unnecessary to renew the water as frequently during cold as during hot weather. The tanks were covered with gauze to exclude dust and prevent any flies from escaping.

Observation 1. Two larvae which had just emerged from the egg measured 0.9 and 0.95 mm. respectively. One was killed and the other measured on successive days. On the second day it was 2.4, the fourth 2.8, fifth 4.0, twelfth 4.3 mm. long. This larva died on the fourteenth day. Average temperature 23–26° C. (July).

Observation 2. Ten young larvae measuring 0.7 to 0.95 mm. were placed in a tank. Two died on the fourth, two on the sixth, one on the tenth day. They measured on the fourth day 1.5 to 2.0 mm., fifth 1.9 to 2.1, sixth 3.0, seventh 3.3 to 3.5, ninth 4.2 to 4.5, thirteenth 5.3 to 6.4, fifteenth 6.4 to 7, eighteenth 7 to 7.5 mm., on which day one pupated, the fly issuing two days later. During the following four days the rest likewise pupated. Temperature during first four days 16 to 19° C., afterwards 23 to 26° C.

The larval stage under the conditions stated lasts from 18 to 21 days. That the larvae attained their full size in the tanks was evident from comparative measurements made upon larvae caught in the open, which soon afterwards pupated. Such larvae measured 6.9 to 7.3 mm., a quite exceptionally large one measured 8.3 mm. Larvae pupate usually when they have attained a length of about 7 mm., though there is a certain degree of latitude in this respect. The rate of development is greatly influenced by temperature¹. A few cool days will greatly retard larval growth. It is doubtless due to lowered temperature alone that

¹ Howard (1900, p. 39) states, without giving the temperature, that the larval stage of *A. maculipennis* lasts 16 days. Grassi (1900, p. 69) gives the duration of the larval stage as 20–22 days during the summer in Italy.

larvae caught in the middle of August had not attained their full growth until November.

Undoubtedly a considerable number of larvae die during the process of development, this no doubt being due in part to difficulties in moulting as also destruction through natural enemies, about which more will be said later. Under natural conditions a very much greater number of small than of large larvae are encountered, and judging from what we see in the laboratory many fully developed larvae die in the attempt to pupate. Of a total number of 834 larvae and pupae caught by one of us at different times in six places in Cambridgeshire, 636 were small larvae (measured up to 4 mm.), and 181 large (measured up to 7 mm.), whereas only 17 pupae were captured. Undoubtedly a number may elude capture, but these figures together with laboratory observation prove that many die off at various stages of growth. The number of pupae caught will naturally be always smaller than that of the larvae because the latter require about eight or ten times as long as the pupae for their development.

Habitat of Larva.

The observations cited in the previous paper have shown that the larvae of *A. maculipennis* and *A. bifurcatus* (those of *A. nigripes* have not been found on account of their rarity) are to be found in pools, ditches, backwaters of rivers, canals, and slowly flowing waters in various parts of Great Britain and Ireland. Exceptionally they are found in water that is impure or brackish. Almost invariably the larvae are found in clear water, and usually such as contains algae or *Lemna*, the latter must however only be present in moderate quantities. The larvae of *Anopheles* certainly prefer waters that are not shaded by trees. Only once, at Streatley, were they found in a shady spot. This has already been noted by Meinert (p. 478), who writes "elle n'aime pas l'ombre des grands bois, mais recherche le soleil et la lumière, ce qu'indique déjà sa fraîche couleur d'herbe." He found the larvae in Denmark between the end of March and October. It is also exceptional to find them in water contained in small receptacles (troughs, fountain-basins and barrels) such as frequently contain the larvae of *Culex*. They were found only nine times with *Culex* larvae, which do not seem to find a sufficient supply of suitable food in the clear water favoured by *Anopheles*. We find that Grassi (4 Oct. 1899, p. 12) has also observed this in Italy. *Anopheles* larvae were present only exceptionally with those of *Culex*

pipiens and *C. annulatus* in foul, greenish water at Maccarese, and impure water in fountain-basins near Rome and at Sermoneta. On the other hand he found them frequently in vessels, barrels, disused cisterns, and surface wells at Grosseto, where he thinks that the insects must have been forced to adapt themselves to altered conditions, the old breeding-pools having gradually been removed by drainage from immediate proximity to the town. In America we find Howard (1900, p. 41) reporting the occurrence of the larvae of *A. punctipennis* (?) in Maryland in a small permanent stream flowing through woods and broadening out into shallows which all contained algae (*Mougeotia* and *Diatoms*) which served as food for the larvae. Larvae of *A. maculipennis* were found in pools about a disused spring in Virginia, the water (8—10 inches deep) containing *Mougeotia* and having a temperature of 18° C. The larvae of an undetermined species were found in pools in an old canal bed, the water being foul, containing algae (*Lyngbya*) and showing a temperature of 25° C. Only empty pupa skins were found in a dried-up surface pool at Washington Barracks at a time when malaria prevailed among the troops. Finally Lazear found *A. punctipennis* breeding in a stone-quarry near Baltimore. Grassi (1900, p. 58) in Italy found larvae of *A. maculipennis* along the margins of relatively deep and large pools during the spring, whilst they frequented shallow waters in summer. Ross, Annett and Austen (1900, p. 17) found *Anopheles* but once in a tub at Sierra Leone, the larvae being absent in fresh-water pools, in mangrove swamps (fish present), as also in shallow puddles liable to desiccation. They were usually present in puddles which continued to hold water, and were not apt to be scoured out by heavy rains. They were also usually present in ditches and pools formed by springs. Whereas *A. costalis* larvae were found everywhere in low parts of Freetown, *A. funestus* was only present in the eastern portion. Stephens and Christophers (July, 1900, p. 45) found the main source of *Anopheles* during the dry season at Freetown to be pools in the rocky beds of small streams, these pools being situated far out into the bush. At times they also found larvae in spring-fed runnels. During the dry season all rock-puddles remain dry for three months.

In the preceding paper¹ the occasional occurrence of *Anopheles* in slightly brackish water is noted. This has also been noted elsewhere, being first mentioned by Grassi (8 June, 1899), who observed it at Metaponto. Christophers and Stephens (Aug. 1900, p. 3) observed *Anopheles* larvae to occur at Accra in brackish pools (0·6% salt), as

¹ *Studies in Relation to Malaria*, Part I.

also in pits about houses. It is interesting to note that they found larvae there in uncovered wells 15—35 feet deep. It is noted in the preceding Paper¹ that the larvae of *Anopheles* were found six times in brackish water in England. In the British Medical Journal (1900, vol. II., p. 400) it has been recently stated that Cook found *Anopheles* in 89 tanks in Calcutta. The larvae are found especially where the water is covered by "green scum," but are less numerous where fish abound. Larvae were also found in stagnant ditches containing waste water from the overflow of stand-pipes. Others do not state that the influence of fish is marked. Rogers (Sept. 1900, p. 348) at Maniktolla Principality (Bengal) found larvae to be very common in numerous large tanks swarming with fish, these tanks being the common breeding-places during the dry season when small pools disappear. He also found larvae in small pools containing fish. The larvae of *A. maculipennis* and *A. bifurcatus* were found ten times by Nuttall, Cobbett, and Strangeways-Pigg in collections of water containing fish in England. There can be no doubt but that the larvae obtain a considerable amount of protection from fish through aquatic vegetation, which usually accumulates about the shallow margins of pools. Near Weybridge on the Thames Dr Cobbett (25 Sept., 1900) found larvae along the banks of the river where the ground was marshy and grass grew out of the water. In numerous little pools more or less connected with the river, which appeared to form suitable breeding-places, no larvae could be found. A considerable difference in the *temperature* of the water in the river and that in the pools was noted, the latter being markedly colder. The weather during the preceding days had been bright and warm during the day, whilst at night it had been cold and sometimes frosty. It is doubtless due to these oscillations of temperature having effect upon the shallow water in the pools that larvae were absent in these. At Byfleet doubtless for the same reason the pools a few yards distant from the river contained no larvae. At Aysgarth in Yorkshire, where no larvae could be found in June and July, larvae were found in September, in grassy pools fed by springs. At this time no larvae could be detected in shallow stagnant pools, containing *Spirogyra*, not fed by springs and subject to considerable fluctuations of temperature occurring during hot days and cold nights. That there is a difference between *A. maculipennis* and *A. bifurcatus* with regard to the seasonal occurrence of the larvae is indicated by Grassi (1900, p. 47) and Ficalbi, who found the larvae of the latter species during midwinter in Italy whilst there

¹ *Studies in Relation to Malaria*, Part I.

were none of *A. maculipennis* to be found. We found the first larvae of *A. maculipennis* in the beginning of May in a ditch; we had not searched for them before this date. On October 20th only two fully developed larvae were found in the Granta after careful search. On the 8th of November Mr Theobald informs us that he found the larvae of this species in all stages of development and in large numbers in a rain-water barrel in his garden. They have been absent all the year round in a couple of pools "where they should be found according to reports," whilst the imago has been nearly always abundant.

It has been claimed by some that the larvae resist desiccation or survive in the mud at the bottom of pools. At first glance this might appear to be supported by two observations we made at Gainsborough and near March, where many fully developed larvae and pupae were encountered in ditches which, according to persons living in the vicinity, had been filled with river water after having been "dry" for 4—7 days. It occurred to us at first that the larvae may have been carried in with the water from the river, as larvae were also found there, but this did not account entirely for the larger number of larvae present in the ditches. The observations of others would indicate that the ditches in question had not been completely dried out. Grassi (17 Sept. 1899) says that he never found larvae nor pupae in moist earth, that is in the absence of water. Christophers and Stephens (1900, p. 20) in Africa found that no large larvae reappeared in a pool which had been dried up for two days and then refilled with rain water. The larvae which did appear issued from eggs which had resisted desiccation for that period. Howard (1900, p. 16) found upon experiment that larvae (presumably of *A. maculipennis*) only survived 24—48 hours in or upon mud from which water had been drawn off. Under natural conditions he observed culicid larvae to retreat with the receding water of a large pool, and to accumulate in vast numbers in the small amount of water in the deeper excavations at the bottom. In this manner the pool only seemed to be restocked with larvae when it increased in size as the result of rains.

To be continued.

EXPLANATION OF PLATES.

Illustrating the Paper of G. H. F. Nuttall and A. E. Shipley on
 "The Structure and Biology of *Anopheles maculipennis*."

PLATE I.

Anopheles maculipennis, male $\times \dots$ (See description in a later number of this Journal).

PLATE II.

- Fig. 1. Egg seen from the side $\times 60$. *a.* the float.
- Fig. 2. Egg seen from the upper surface $\times 60$. *a.* ridge of air-chambers which acts as a float.
- Fig. 3. Very young larval stage $\times 60$. *b.* brush, *c.* antennae, *d.* palp of 1st maxilla, *e.* thorax, *f.* stigma.
- Fig. 4. Fully grown larva $\times 16$. *b.* brush, *c.* antenna, *d.* palp of maxilla, *e.* thorax, *f.* stigma, *g.* palmate hairs, *h.* tergum, *i.* anal papillæ.
- Fig. 5. A palmate hair, highly magnified.
- Fig. 6. Flabellum or flap which overhangs the base of certain thoracic hairs.
- Fig. 7. Side view of head of a fully-grown larva. *b.* brush, *c.* antenna, *d.* palp of maxilla, *m.* hooked hairs at edge of maxilla, *p.* median tuft of hairs, *r.* thickened rim of chitinous covering to head, *s.* large, feathered hairs which overhang head, *t.* mandible, *u.* larval eye, *v.* eye of adult forming above and behind *u.*
- Fig. 8. Ventral view of head of a fully-grown larva. *b.* brush, *c.* antenna, *d.* palp of maxilla, *j.* stout hairs of mandible which arrange the brush, *k.* teeth of mandible, *m.* hooked hairs at edge of maxilla, *p.* median tuft of hairs, *q.* the "under-lip" of Meinert, or metastoma, *r.* thickened rim which passes into the soft tissue of the neck.
- Fig. 9. Side view of last segment, showing the four anal papillae and the dorsal and ventral hairs.
- Fig. 10. Side view of late pupal stage, *f.* the stigma opening at end of trumpet-like projections.
- Fig. 11. Upper or oral view of mandible. *j.* stout hairs which run through the brushes, *k.* teeth, *l.* hairs projecting inwards to mouth.
- Fig. 12. Upper or oral surface of first maxilla, *d.* palp, showing the three spines and the plate. *m.* hooked hairs at edge of maxilla, *n.* hairs lining oral surface of maxilla.
- Fig. 13. Dorsal view of the third abdominal segment of a larva about half grown, to show arrangement of hairs, *z.* palmate hair, *o.* long, balancing hair.
- Fig. 14. Ventral view of the same. *o.* long, balancing hair.
- Fig. 15. Stigmatic apparatus seen from above, *f.* stigma, *w.* valve or flap which folds down and breaks the continuity with the surface-film when the animal sinks, *y.* chitinous skeleton which supports the whole apparatus.

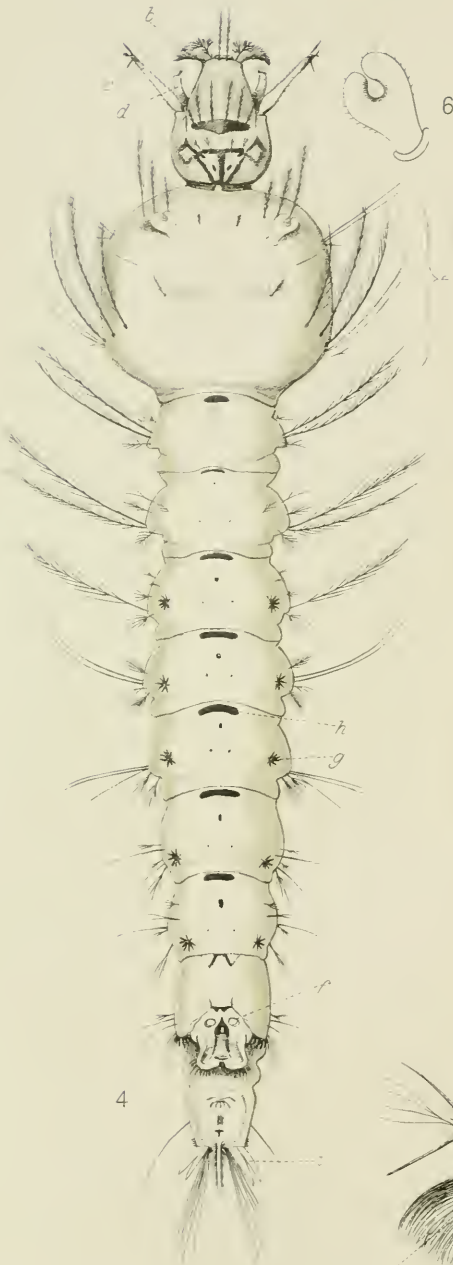
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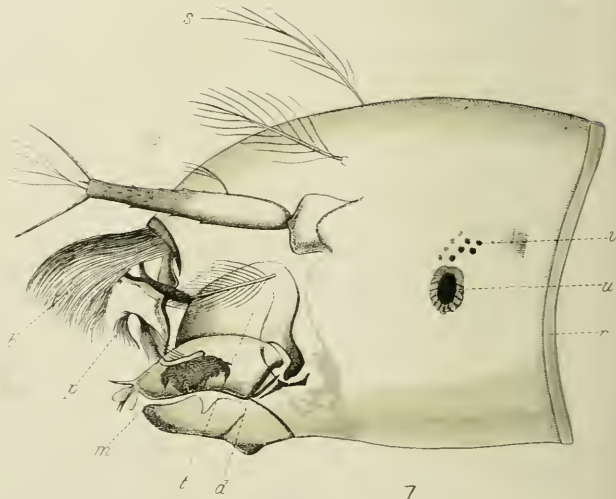
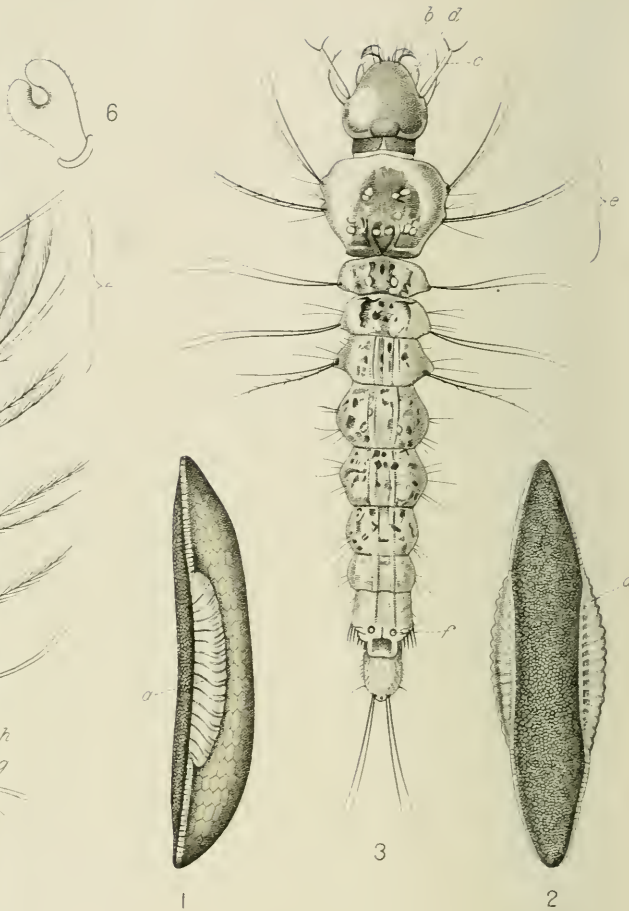
ANOPHELES MACULIPENNIS ♂

IMAGEN

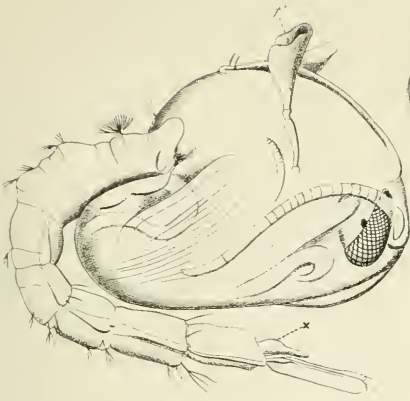
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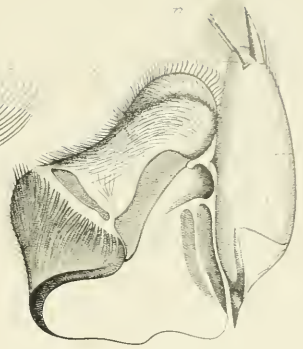
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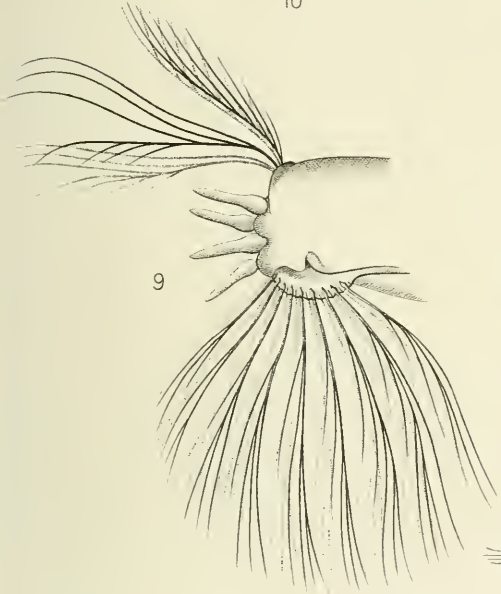
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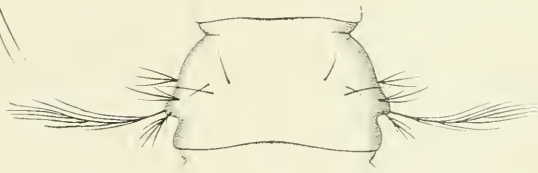
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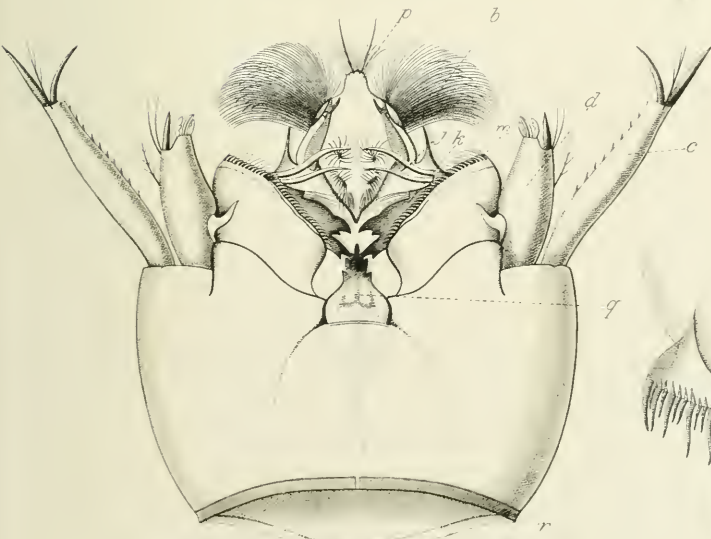
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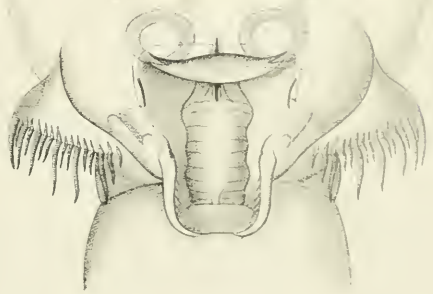
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PATHOGENIC MICROBES IN MILK.

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MILK, as every bacteriologist knows, is not only a universal and excellent food-stuff for human beings, but a medium admirably adapted for the growth and multiplication of microbes. For the latter reason milk deserves every attention at the hands of the hygienist, it being incontestably established that it may serve as a vehicle of disease agents. How well natural milk is adapted to this purpose, viz. to serve as nutritive medium for bacteria, is clear from its alkaline condition, and from its containing all ingredients required for the growth and multiplication of bacteria: proteid, fat, carbohydrate, and a large percentage of the essential mineral matters. It is a matter of common experience that all milk, however carefully it may be collected, however clean and aseptic may be the vessels into which it is received, will on standing, or after being handled in the way usual between collection and distribution, be found to teem with various kinds of bacteria. This fact is confirmed by the bacteriological examination of the milk sold in London shops, milk which, normal though it may be in appearance, chemical analysis, and taste, is usually found to contain hundreds of thousands of bacteria per cubic centimeter; bacteria which belong to various species and some of which when grown separately in sterile milk cause rapid changes and alter profoundly the character of the milk, *e. g.* *Bacillus lactis*, *Proteus vulgaris*, *Bacillus coli*, *Bacillus mesentericus*, spores of *Bacillus enteritidis*, etc. If allowed to stand, the milk containing the above mixture of bacteria exhibits even at ordinary temperatures, but in a more marked degree at temperatures of 70° F. and above, those profound changes which are popularly expressed as "going bad," changes caused by the rapid multiplication of one or other of the above microbes. Thus, for instance, if different samples of

milk received and brought in a sterile vessel from a shop be placed in the incubator at 37° C. the next day, or at the latest, after two days it may be completely clotted and sour, due to the growth and activity of *Bacillus coli*, or it may be decomposed by *Proteus* or *Bacillus mesentericus*, or it may be full of gas, clotted with a large amount of clear whey caused by the growth of the anaerobic *Bacillus enteritidis sporogenes*—the layer of cream on the top of the milk insuring something approaching anaerobiosis.

The enormous number and nature of bacteria present in ordinary seemingly perfectly normal and wholesome milk prove how easily milk becomes the receptacle of extraneous bacteria derived from dust and utensils, and how readily these multiply therein. When one considers the conditions under which milk is received from the udder, the nature and amount of handling it is subject to before it reaches the consumer, further, that the methods used in these manipulations are far from preventing—if anything the reverse is the case—the milk receiving extraneous matters abounding in microorganisms, we cannot wonder that milk as a rule does contain such multitudes of bacteria. Nor can we wonder that milk readily becomes a vehicle for infectious diseases like typhoid, diphtheria, and scarlet fever, if in the course of the long way between the cow and the consumer access is given to it for the specific microbes of these diseases.

Not only as a receptacle of extraneous microbes, both pathogenic and non-pathogenic, but also as a receptacle of microbes derived direct from the cow or the cow's udder, does milk deserve special attention, and in this article I will limit myself to certain pathogenic microbes which were found in samples of milk collected and analysed at the instance of the Medical Officer of the London County Council during the first months of last year. These samples were taken by an inspector in sterile glass-stoppered bottles from milk churns sent from country farms to the principal stations in London, before being handed over to the agents. Immediately after filling, the bottles were carefully stoppered, sealed, tied and brought directly to the laboratory. The bacteriological analysis was undertaken chiefly with the view of seeing whether or not any sample of the milk contained the tubercle bacillus, but in the course of the inquiry some other microbes were detected now and again, which on account of their specific pathogenicity to animals, at any rate, deserve consideration.

The Bacillus tuberculosis.

The statements by different observers as to the percentage of occurrence of the tubercle bacillus in cows' milk are of so divergent a character that it is impossible to explain them by different methods used in the analysis, or by faulty diagnosis. I am rather inclined to assume that the cows were less frequently affected with tuberculosis when the milk yielded a low percentage, and more affected where a high percentage was obtained. I think this is the more likely because no one amongst those observers who have published their analyses could be assumed not to have undertaken all and every test necessary for a reliable diagnosis, and I would therefore refuse to admit the suggestion that has been made¹ that some of the published high percentages probably include samples which did not produce real tubercle in the experimental animals but produced pseudo-tuberculosis. If such an explanation were a good one it would imply that the observer omitted some of the most important tests for his diagnosis, viz. the demonstration of the real tubercle bacillus in the deposits of the animals experimented upon. In this I am assuming that the microscopic specimens (cover-film specimens) of the deposits have been suitably prepared. Under suitably prepared specimens I understand not merely that the films made from the deposit were stained in fuchsin and treated with dilute mineral acid, and after this counter-stained with methyl-blue, thus showing bacilli which retained the pink coloration; for these are manipulations which admit of great variations, variations which may, and which as a matter of fact do, affect the result. By suitable preparations I understand that the cover-films are placed in carbolfuchsin solution (Ziehl) and heated over the flame till the stain boils; the films are now washed in water to remove the excess stain, then washed thoroughly in 33 p.c. nitric acid; a treatment of 10—15 seconds being sufficient to remove all red as far as naked eye inspection is concerned; then washed in water, whereby a little of the red tint reappears. Now the films are placed in methyl-blue anilin-water for $\frac{1}{4}$ of a minute, washed well in water, dried, and mounted in balsam. If real tubercle bacilli are present they appear as bright pink, slender bacilli of a distinctly cylindrical shape, and showing the well-known segregation of their protoplasm.

I am not aware of any bacilli belonging to the acid-resisting forms

¹ Annett, *Thompson-Yates Laboratories Reports*, Vol. II, p. 32, 1898-1899.

hitherto described as capable of simulating the tubercle bacilli of tubercular deposits, which under this mode of staining present the above well-pronounced acid-resisting qualities and morphological characters. Too weak acid, insufficient time in the acid, or insufficient counter-staining may bring forth a picture simulating acid-resisting bacilli, but I have never found yet that washing for 10—15 seconds in 33 p.c. nitric acid and counter-staining for $\frac{1}{4}$ minute in methyl-blue anilin-water did not reveal and differentiate the true tubercle bacilli; and if under this treatment the films show the well-known slender cylindrical bacilli with segregated protoplasm they can be relied upon to be the true tubercle bacilli.

A no less important item in framing the diagnosis is that of culture. I have not found the least difficulty in obtaining the characteristic colonies of the tubercle bacilli on the slanting surface of solidified horses' serum if this surface is inoculated with a fair quantity—of course under the usual precautions—of the caseous or purulent deposits of the omentum, pancreas, lymph glands or spleen of the experimental animal. By the end of 8—10 or 12 days the first indications of growth are noticed, and the developing colonies can after several more days be used for the preparation of films and for experiments on animals.

Besides these tests, the nature and progress of the disease in the inoculated guinea-pigs are of importance, as also the histological character of the tubercular deposits in the viscera of the experimental animal. As in most cases time is an important factor, I invariably inoculate a large amount of the sediment of the milk sample into two guinea-pigs: Animal I. receives subcutaneously into the groin half of the sediment of about 250 c.c. of the original milk distributed in a few c.c. of the milk, and Animal II. receives the other half intraperitoneally. By inoculating the two animals in different ways the test is more apt to lead to a successful result, it having frequently been observed that more especially the animals which are inoculated subcutaneously may die of acute septicaemia. It might be added that not one of 120 samples which I used for the inoculation (240 animals) produced acute death in both guinea-pigs. Had I relied upon the result of the subcutaneous inoculation of a single guinea-pig a considerable percentage of the tests would have failed.

The various statements as to the percentage of true tubercle bacilli in the milk of proved tubercular cows as demonstrated by animal experiment vary between 14 and over 71·4 p.c.; Bang 14 p.c.; Hirschberger over 50 p.c.; Ernst 28·5 p.c.; Rabinowitsch and Kempner 71·4 p.c.;

Boyce found 6—8 p.c. of 'town' milk and 17 p.c. of 'country' milk to contain tubercle bacilli.

Out of 100 samples of 'country' milk which I analysed, seven proved to contain the true tubercle bacillus. Amongst the 93 remaining samples there was one which was derived from a cow that, according to the veterinary inspector, was affected with tuberculosis, but its udder was free from disease. The milk of this animal did not contain the tubercle bacillus. The proof in the above seven cases was furnished (*a*) by the result of animal experiment: the disease—inoculation tuberculosis—was quite typical in its progress and pathology, and the deposits contained an abundance of typical tubercle bacilli—typical as regards aspect, size and staining; and (*b*) by culture on horses' serum, the culture being obtained from the deposits of the experimental animal. Tubercle bacilli could only be detected in one of the seven samples, films having been prepared in the usual manner from the milk sediment. On the other hand, the intraperitoneally as also subcutaneously injected guinea-pigs developed characteristic lesions of the lymph glands and viscera in the course of 3—5 weeks.

An important series of observations which were carried out for the Local Government Board proved that tubercle bacilli grow well in milk kept at 37° C. When sterilised milk is inoculated with tubercle bacilli derived from a culture on serum or from a tubercular deposit of the omentum, spleen, or lymph gland of a guinea-pig it shows, after a fortnight and later, a good growth of tubercle bacilli in the deeper layers, the milk and layer of cream remaining macroscopically unchanged. When a little of the bottom layer is removed by means of a capillary pipette great numbers of small and large clumps of typical (cylindrical slender 'granular') tubercle bacilli are found, these clumps being composed, just like the colonies on the surface of serum, of wavy, branching and reuniting strands and festoons of the tubercle bacilli. After four to six weeks the number of small and large clumps of bacilli present in the deeper layers is of course greatly increased. Such milk cultures prove to be highly virulent on inoculation into guinea-pigs, distinctly more virulent than the original materials (as shown by control experiments) with which the milk was inoculated. This increase of virulence through cultivation in milk is strikingly shown by inoculating sterilised milk from a glycerine-agar culture which has lost its virulence as the result of cultivation through many generations upon glycerine-agar. I possess glycerine-agar sub-cultures which have been carried on from generation to generation on this medium for over ten years.

The growth is very rapid and characteristic, *i.e.* folded crinkled membrane on the surface of the fluid of condensation and on the slanting surface of the glycerine-agar; and by staining, the culture can be shown to be composed of typical acid fast tubercle bacilli. When transferred to serum (slanting surface) the culture forms characteristic colonies of tubercle bacilli. But when the above glycerine-agar cultures are tested on the guinea-pig it is found that even large quantities—one-third to one-half of a culture—the whole slanting surface being covered by the growth) injected subcutaneously or intraperitoneally fail to produce any effect, not even a local one. If, however, sterilised milk be inoculated from such a non-pathogenic glycerine-agar culture, it is found that after incubation of even a week good growth has taken place, better still after a fortnight. If then from such a milk culture, say after two, three, or more weeks, guinea-pigs are inoculated subcutaneously or intraperitoneally each with several drops of the milk, the result is in a large percentage positive. Some animals do not show any result, but the majority develop tubercles which are crowded with tubercle bacilli. Animals injected subcutaneously in the groin develop, in the majority of instances in the course of a month, distinct swelling and caseo-purulent tubercles of the inguinal glands; those injected intraperitoneally show caseo-purulent tubercles in the omentum and pancreas, as also in the spleen; a small number of guinea-pigs developed general and fatal inoculation tuberculosis in the course of two, three or more months. The tubercles in all the positive cases show in stained film specimen crowds—chiefly in clumps—of acid fast, typical tubercle bacilli, and culture on serum, which was practised in all positive instances, yielded readily copious and pure growths of the tubercle bacilli. From this I think there can be no doubt that by growing even highly attenuated tubercle bacilli in milk the pathogenic action can to a large extent be restored, though it must be added that in the majority of instances the inoculation of such milk culture produces only local tubercle, and further that only in a small percentage did it lead, after long periods, to general fatal tuberculosis.

Pseudo-tubercle.

Amongst the hundred samples of country milk analysed, as above mentioned, eight contained the *Bacillus pseudo-tuberculosis* as proved by the experimental results. The guinea-pigs injected subcutaneously or intraperitoneally with the sediment of these eight samples developed,

in the course of three to four weeks, caseo-purulent nodules in the inguinal lymph glands (subcutaneous injection), caseo-purulent nodules in the omentum and pancreas (intraperitoneal injection), caseous or purulent nodules in the spleen, pelvic lymph glands, liver, and besides, in several instances, in the lungs. The caseous and purulent matter of the above lesions did not contain the acid fast tubercle bacilli or any other acid fast microbes, but contained an abundance of the relatively thick, rounded, short, oval bacilli (lying often within the pus cells and contained in abundance in the necrotic tissues) which in their cultural character, distribution and action are identical with the classical *Bacillus pseudo-tuberculosis* first cultivated by A. Pfeiffer¹, and carefully investigated by Preisz² and others. I myself have described them³ as occurring in sewage, and in water polluted with sewage.

The *Bacillus pseudo-tuberculosis* resembles the *Bacillus coli* in size and shape. On gelatine and agar the colonies resemble and grow nearly as fast as those of *B. coli* or colilike microbes, though the resemblance ceases here, for in broth, sugar gelatine, milk, and on potato, the characters are altogether different from those of *B. coli* or colilike microbes. Milk remains unaltered, the *B. pseudo-tuberculosis* forming no acid, on the contrary it forms alkali. It forms no indol. Its action on the guinea-pig, rabbit and mouse is definite, both on subcutaneous and intraperitoneal infection. By feeding guinea-pigs with the culture it produced caseous purulent deposits in Peyer's glands, the mesenteric glands, omentum, pelvic glands, spleen, liver and lungs. The action of recent cultures is far more rapid than that of the tubercle bacillus.

It is generally recognised that the *Bacillus pseudo-tuberculosis* of A. Pfeiffer represents a well-defined species, well-defined by its morphology, cultural characters and action. It seems therefore greatly to be regretted that some observers apply the name of *Bacillus pseudo-tuberculosis* to an altogether different species—different in morphology, culture and experiment—of bacilli. Petri, Rabinowitsch, Möller and others have described certain acid fast bacilli occurring in milk and butter which, on injection into guinea-pigs, cause disseminated caseous deposits in the viscera, and which they describe as pseudo-tuberculosis, a process as slow as that of true tubercle and only limited to the guinea-pig. The same applies to Dr Annett⁴, who follows Rabinowitsch in

¹ Ueber die bacilläre Pseudotuberculose bei Nagethieren, Leipzig, 1889.

² Annales de l'Institut Pasteur, 1894, No. 4.

³ Centralbl. f. Bakteriologie, xxvi. No. 9.

⁴ Thompson-Yates Laboratories Reports, 1898-1899, Vol. II, p. 33.

accepting the acid fast non-tubercle bacilli of milk and butter as *Bacillus pseudo-tuberculosis*, and their action on the guinea-pig (subcutaneous or intraperitoneal) as pseudo-tuberculosis. This can only lead to confusion, and I think the name of *Bacillus pseudo-tuberculosis* should be reserved to the organism described by A. Pfeiffer and others.

It will then be understood that the pseudo-tuberculosis and the *Bacillus pseudo-tuberculosis* which I mentioned as having been met with by me in eight of the one hundred samples of country milk is the non-acid fast microbe which was first isolated and described by A. Pfeiffer, and which I met with also in sewage and in sewage polluted water; which is pathogenic to guinea-pig, rabbits and mice, and which on inoculation and feeding produces in the guinea-pigs more rapidly than the true tubercle the above-mentioned caseo-purulent deposits in the lymph glands, in the abdominal, and further in the thoracic viscera¹.

Bacillus diphtheriae.

Amongst the 100 samples of country milk referred to above, one produced on subcutaneous injection into the groin of the guinea-pig a swelling of the inguinal lymph glands—the intraperitoneally injected guinea-pig remaining quite well. By the fifth day the inguinal glands of the first guinea-pig were found swollen to about the size of a filbert and surrounded by soft oedematous tissue. It presented the following appearances at autopsy: about the seat of inoculation the subcutaneous tissue was oedematous and streaked with blood. The inguinal glands were enlarged, firm and deeply congested. Film specimens which were made of the juice of the incised gland, and stained, showed numerous bacilli closely resembling the diphtheria bacilli in size and shape. Cultures made on agar and ascites-agar brought forth numerous colonies of a pure culture of the *Bacillus diphtheriae*. A broth culture was made from one of these colonies and after 48 hours' incubation at 37° C. showed the characters of a diphtheria culture, forming acid. One quarter of a cubic centimeter was injected subcutaneously into the groin of a medium-sized guinea-pig with the result that the animal died in 36 hours with haemorrhagic tumour in the groin and deep congestion of the viscera. Films and cultures made from the fluid of the tumour showed the diphtheria bacilli in pure culture. Stained

¹ Reports of the Medical Officer of the Local Government Board, 1899–1900; and *Centralbl. f. Bakt. und Infekt.* Vol. xxvi. No. 9.

according to Neisser's method the bacilli gave a positive result like true diphtheria bacilli.

A final proof that we were dealing with the true diphtheria bacilli was furnished by the following experiment :

Of a 48 hours' old broth culture, $\frac{1}{4}$ c.c. was injected into the groin of a medium-sized guinea-pig *a* (weight 306 grammes); another medium-sized guinea-pig *b* (weight 302 grammes) received a mixture of $\frac{1}{4}$ c.c. of the same broth culture and $\frac{1}{10}$ c.c. of Burroughes and Welcome's diphtheria antitoxin. The result was striking: guinea-pig *a* was dead in 36 hours with the characteristic tumour, guinea-pig *b* had no tumour at any time and remained lively and well.

From these observations it is justifiable to conclude that the bacilli in question obtained from the above sample of milk were the true diphtheria bacilli.

After these results had been obtained inquiry was set on foot as to the derivation and distribution of the milk. This could be done readily because the farm from which the milk was derived was known. The dealer who received the milk and the locality in which the milk, of course mixed with other milk, had been distributed were known. But nothing suspicious could be found; the farm and its employees were in all sanitary respects correct, and no case of diphtheria could be discovered amongst the houses to which the milk was delivered, either directly from that farm or after mixing with other milk.

Whether owing to the small number of diphtheria bacilli originally present in the milk or perhaps to their lesser virulence, or owing to the possibility that the consumers of the milk had all healthy throats and therefore were less susceptible to infection, no cases of diphtheria could be referred to that milk, must remain undetermined; the fact remains, that the sediment of the milk produced, by subcutaneous injection into the guinea-pig, a disease which could only be regarded as diphtheria of a somewhat subnormal type, considering that it took the better part of a week to develop; this would also point to the number of diphtheria bacilli originally present in the milk being very small. I need scarcely say that any accidental contamination with diphtheria bacilli in the laboratory either of the milk treated or the instruments used, is altogether excluded, there having been no diphtheria work done for a considerable time, certainly for more than half a year previously.

Bacterium diphtheroides.

The secretion of an indurated quarter of the udder of a cow was collected and the milk was submitted to bacterioscopic analysis. The induration was of a chronic nature and the secretion was of the nature of thick creamy pus. The veterinary inspector declared the induration to be of the nature of tuberculosis, but neither the microscopic examination of the pus nor the experiments of injection into guinea-pigs confirmed the diagnosis. The microscopic examination of the secretion revealed the presence of a conspicuous number of bacilli, singly, but more frequently in larger and smaller clumps, which had a certain resemblance in their shape and size to diphtheria bacilli; amongst them there were clubbed forms. By injection into the peritoneal cavity or subcutaneously into the groin, sub-acute abscess was produced, containing thick yellowish-white 'granular' grumous pus. This abounded with large and small masses of the microbe.

The pure culture of the microbe injected in small quantities subcutaneously or intraperitoneally causes local abscess in the course of from one to two weeks. This abscess after subcutaneous injection into the groin comprises the inguinal glands and the surrounding tissue, and reaches after three weeks the size of a pigeon's egg. After intraperitoneal injection abscesses are produced on the omentum, the pancreas or around the kidney.

Pure cultures were easily obtained both from the original cow-secretion as also from the pus of the abscesses in the guinea-pig. In the latter case, as mentioned above, the microbe abounds to an enormous extent, so much so that the 'granules' of the pus are almost entirely made up of the bacterium. The microbe does not stain readily in the ordinary dyes, but it stains easily and well by means of Gram's method: 1 minute gentian violet, 4 minutes iodine iodide of potassium.

Although in shape and size this bacillus belongs to the group of the diphtheria bacillus its cultural characters readily differentiate it from the latter and from the known diphtherioid bacilli, *e.g.* bacillus of Hoffmann, and the group of Xerosis bacilli.

In the first place it does not grow on gelatine at 21° C., it does not grow below 25° C., it shows very little or no growth in ordinary nutrient bouillon at 37° C. On agar and glycerine agar at 37° C. its growth is very slow and limited, the colonies do not appear before the third day, and then are small grey dots, which on subsequent days enlarge to

circular plates with a thick, dark, granular centre and a greyish, thin translucent margin, which latter is somewhat irregular and angular.

In stab agar there is no growth in the depth, only on the surface of the stab is there a small, flat, circumscribed, grey plate. The growth of the microbe in milk and solidified blood serum is, however, very characteristic, and by it our microbe is easily distinguished from all other known diphtherioid bacilli; viz., it coagulates milk at 37° C. and forms acid: litmus-milk becoming red; beginning with the third day the milk (as also the litmus-milk) separates into the top cream, a chief middle layer of clear whey and at the bottom the white coagulated casein. On the slanting surface of solidified blood serum the microbe grows as small, round, granular colonies; these make their appearance on the third day and are recognisable by the depression (liquefaction) of the serum; on the third and fourth day the surface of the serum is uniformly pitted, each pit being a depression (liquefaction) with a small colony in its depth. Gradually and slowly, as growth proceeds, the serum becomes liquefied.

Comparatively speaking the microbe dies off rapidly on agar and glycerine-agar, but retains its vitality longest on serum; I have succeeded in obtaining good cultures on this medium, even after several weeks' transference.

As mentioned above, owing to its shape, I have called the microbe *Bacterium diphtheroides*¹, it is, however, in respect of staining, in its cultural characters and in its action on the guinea-pig easily differentiated from the known diphtherioid bacilli. Formation of local abscess occurred in all guinea-pigs subcutaneously injected, whereas only about half of the animals develop abscesses of the abdominal viscera after intraperitoneal injection.

Streptococcus radiatus (pyogenes).

A large number of observations have been reported regarding the occurrence of streptococci in the diseased udder of cows. I have myself found and isolated from purulent secretions of the udder of different milch cows: *Streptococcus pyogenes*, *Streptococcus brevis*, and *Streptococcus longus*; in these cases the streptococci were present abundantly in the purulent matter and in masses, particularly in some of the purulent matter *Streptococcus pyogenes* and *Streptococcus longus* occurred in great numbers and in aggregated masses.

¹ *Centralbl. f. Bakt. und Parasit.*, Vol. xxviii. Nos. 14, 15.

But there have been also described as *Streptococcus mastitidis*, a specific microbe causing a specific contagious purulent inflammation of the udder. Nocard and Mollereau¹ described and isolated this microbe first and proved by inoculation in cows and goats that it is capable of producing mastitis. In Germany the disease is known as 'Gelber Galt,' and the streptococcus of the French observers was isolated in this affection by Eisenberg, Adametz, Zschokke and others.

Amongst the secretions of diseased udders submitted to me for bacterioscopic analysis (with the object of testing whether they contained tubercle bacilli) there was one which was not of the character of purulent matter, but was a thin serous exudation with fibrin and blood. Injected into the subcutaneous tissue of the groin or into the peritoneal cavity of the guinea-pig it caused acute purulent inflammation. The serous fibrinous exudation of the udder, and more especially the purulent exudation in the guinea-pig, contained streptococci, which in culture proved to belong to one and the same species, and to possess characters not coinciding with those of hitherto described species. In the purulent exudation of the guinea-pig our streptococcus occurs in very large numbers, as shorter or longer chains, isolated or in small aggregations or forming dense convolutions and big clumps. A small amount of the culture injected into the groin of the guinea-pig produces in a few days, in the great majority of instances, abscess. The streptococcus stains easily in ordinary dyes; it stains well by Gram's method; it measures 0.6—0.8 μ .

The microbe grows in a characteristic manner on the surface of gelatine: after a few days' incubation it forms grey, translucent, round colonies; these show a thicker, dark, granular centre, from which radiate densely aggregated fine striæ to, and also here and there beyond, the margin, whereby the outline is slightly crenate and toothed. This character on gelatine distinguishes it from *Streptococcus pyogenes* and for this reason I proposed the name of *Streptococcus radiatus* (*pyogenes*). The gelatine is at no time liquefied—which character distinguishes it at once from the *Streptococcus radiatus* (non-pathogenic) of Flügge. On the surface of agar our microbe forms round flat discs with thicker centre and translucent periphery; the margin is also here and there irregular and crenate. It grows well in the stab both in gelatine and in agar, the line of inoculation being marked by a row of dark (white in reflected, brownish in transmitted light) separate granular colonies; on the surface of the stab there is very little growth.

¹ *Annales de l'Institut Pasteur*, 1. p. 109. 1887.

In milk (at 37° C.) it grows well, the milk remaining fluid (unlike the *Streptococcus mastitidis*), though the use of litmus-milk shows that acid is formed. In alkaline broth it grows well at 37° C., and in this medium it is readily distinguished from *Streptococcus pyogenes*, the broth remaining clear, but at the bottom are formed greyish-white, flocculent masses, just like those produced in broth by *Streptococcus conglomeratus scarlatinae*.

On solidified serum the growth is very rapid and resembles that on agar, except that on serum the contrast between dark centre and translucent broad margin is more pronounced; the serum is not liquefied.

The cultures lose their vitality rapidly, so that before the end of the week new transference has to be made in order to keep the cultures going. The microbe lives longest in gelatine stab-culture. The characters described, particularly those exhibited on the surface of gelatine, in broth and on serum, indicate, that our *Streptococcus radiatus* differs markedly from *Streptococcus pyogenes*, as also from those hitherto described of the diseased udder; its pyogenic action on the guinea-pig distinguishes it also from the *Streptococcus mastitidis* of Nocard and Mollereau. Although in broth it resembles *Streptococcus conglomeratus scarlatinae*, it differs from this latter by the character of the colonies on gelatine, agar and blood serum, and by the fact that it does not curdle milk.

The two pathogenic microbes, *Bacterium diphtherioides* and *Streptococcus radiatus pyogenes*, mentioned in the foregoing pages although obtained from secretions of diseased udders, may and probably do find access to the milk obtained from the rest of the udder, since it is the usual practice not to discard the milk of the three apparently sound quarters if one quarter appears to be diseased, and for these reasons, I think, these microbes deserve a place amongst the pathogenic microbes in milk.

Pathogenic Yeast in Milk.

I now propose to describe a microbe which was obtained from a sample of country milk which in all respects appeared normal, but which on subcutaneous injection into the guinea-pig produced a chronic and peculiar pathological condition.

The history of the disease in the guinea-pig is as follows: with the sediment of a sample of 'country' milk (this being one of the samples

delivered by the Inspector of the London County Council) two guinea-pigs were injected: one subcutaneously, the other intraperitoneally. After three weeks both animals were killed. The intraperitoneally injected guinea-pig was found at autopsy to be quite normal, the omentum, pancreas and all viscera being free of any disease.

The subcutaneously injected guinea-pig showed a big tumour in the groin of the inoculated side, this tumour included the swollen hyperæmic lymph-glands; when cut into, a quantity of thick, greyish, viscid fluid was obtained, which on microscopic examination showed a few red blood corpuscles, numerous pus-cells and crowds of yeast-cells of different sizes: some not larger than a red blood corpuscle, others twice and thrice as big; there were also present numerous longer and shorter moniliform cylinders, in which the varicosities corresponded to the outlines of individual yeast-cells; in some of these cylinders the terminal element was much enlarged, pear-shaped or club-shaped. The yeast-cells were met with singly and more frequently in masses held together by a gelatinous interstitial substance: on staining they showed a thick homogeneous capsule. In the fresh state many of the large yeast-cells showed within the membrane a clear marginal plasma, in the centre a mass of granular substance. By the ordinary aniline dyes the cells and cylinders stained very easily. Most of the yeast-cells are spherical, some, the larger ones, oval or pear-shaped. There was no difficulty in finding such as showed distinctly the process of gemmation. The tumour did not contain any bacteria and no tubercle bacilli could be detected.

Cultivations made on agar and glycerine-agar (at 37° C.) and on gelatine (at 20° C.) proved that the juice of the above tumour contained only yeast cells, these forming innumerable colonies; there were no colonies of bacteria.

Inoculations were made of a number of guinea-pigs and rabbits with the juice of the above tumour, and subsequently many inoculations were made with the sub-cultures of the above yeast-cells, and I will here give a summary of the results both of the animal experiments, as also of the cultural characters of the yeast.

First as to the animal experiments:

(a) Subcutaneous injection in the groin with the matter of the tumour from the above guinea-pig or from other subsequently inoculated animals causes tumour of the inguinal lymph-glands of the injected side. This tumour shows itself by the end of the week as a soft nodule about the seat of inoculation. By the end of two weeks several nodules

are noticed, some in the groin, others, larger ones, extending towards the back—sacral region. The animals either die about the end of the second week or the tumours change into abscesses. In the first case the autopsy shows that the tumour consists of a mass of firm, gelatinous tissue with more or less haemorrhage in it; in the other case the abscess contains thick, viscid grumous purulent matter. But in all instances the matter of the tumour or of the abscess is crowded with yeast-cells of exactly the same description as in the first case: viz. spherical cells varying in size from that of a red blood corpuscle to that twice or thrice as big; the great majority are spherical, some few large ones are oval or pear-shaped, while others are moniliform cylinders.

Some of the subcutaneously injected guinea-pigs developed in the course of three weeks an enormous tumour—as large as a hen's egg—in the groin and extending on to the thigh and sacral region; the animals died between the 19th—25th day. The tumour on cutting into it looked like blood-streaked bacon in the peripheral part, like a semi-fluid jelly in the central part. In all parts continuous masses of yeast-cells were found.

(b) After intraperitoneal injection the guinea-pigs as a rule die about the end of two or three weeks (14—20 days) seldom later; at autopsy numerous small and large whitish nodules are observable in the omentum, pancreas, and sometimes also in the spleen. The mucous membrane of the stomach and large intestines shows numerous whitish spots surrounded by haemorrhages; the haemorrhages and whitish spots are particularly conspicuous in the peritoneum around the ovary in females, and the testis and epididymis in males. But what is very remarkable is the circumstance that in many such animals the stomach and large intestine are enormously distended by gas; the lungs show petechiae and look almost emphysematous and full of closely placed gas-bubbles.

All the above whitish nodules contain besides leucocytes great numbers of the yeast-cells as is shown by cover-film specimens and culture, and sections through the organs demonstrate the presence of the yeast. Here also amongst the single and aggregated yeast-cells there occur the moniliform cylinders above mentioned.

In addition to the above studies upon the distribution of the yeast-cells in the diseased organs, cultivations were made also of the heart's blood, both of subcutaneously and intraperitoneally injected guinea-pigs that died spontaneously, and it was found that yeast-cells

were present also in the blood; in some cases the culture was negative, in others a drop of blood yielded three, in another eight, and in one case as many as 28 colonies.

Mice are susceptible to infection. After subcutaneous injection with culture into two mice, one died within 48 hours; all the viscera were found on autopsy to be deeply congested, and the heart's blood yielded colonies of yeast-cells on cultivation. The second mouse was ill after two days; being quiet, cuddled up with curved back; coat rough, eyes closed, and not feeding; it remained in this condition off and on for about a fortnight. Then it became again lively, fed well and completely recovered.

The only experiments on rabbits hitherto made consisted in the intravenous injection of two animals (Nos. 1 and 2) with salt emulsion of an agar-culture of the yeast. The animals appeared quite well and fed well for the first fortnight; then they became quiet and refused food; by the end of 24 days both animals showed great weakness in the hind limbs; one rabbit, when trying to walk, dragged the hind limbs after it; the breathing was laboured. The other developed the paraplegia a week later. Rabbit No. 1 died after 31 days, the other, No. 2, after 39 days. In both cases the bladder and intestines were found at autopsy to be much distended. The chief changes, however, were in the cord: the lower dorsal and lumbar cord being greatly congested both in its substance and membranes; yeast-cells were found in these regions, both in the cord and its membranes.

The cultural characters are these: The microbe grows well on alkaline gelatine at 20° C., on alkaline agar at 37° and on blood serum, in milk at 37° C., whereas it grows feebly in ordinary alkaline bouillon. It grows much faster and more copiously on grape-sugar-gelatine, on grape-sugar-agar and in grape-sugar-bouillon. It grows better and more vigorously on alkaline than on neutral or acid media; it grows well on the surface of solid media, but shows only feeble or no growth in the depth (stabcultures). It does not produce fermentation (gas) in any medium, be it growing on the surface or in the depth, be the medium gelatine, agar, or bouillon, to which grape-sugar has been added. It does not produce any fermentation in beerwort-gelatine.

The colonies on ordinary alkaline nutrient gelatine are thick and rounded, moist looking and raised in the centre; white in reflected, brown and granular in transmitted light; on sugar-gelatine the colonies grow more rapidly, are larger and thicker, and with time assume a light

yellow colour and slowly liquefy the gelatine into a thick, turbid, syrupy mass; such liquefaction does not occur at all or only after many weeks' growth on ordinary gelatine. On glycerine-agar and on sugar-agar the microbe forms in a few days a thick, smeary, viscid growth, gradually assuming a yellowish colour; on ordinary nutrient agar the growth is less copious and whitish in reflected light. In sugar-broth the microbe forms a white powdery sediment leaving the broth clear; the same is the case in ordinary bouillon, but to a much more limited degree. In milk and litmus-milk the microbe grows well at 37° C., the milk remaining fluid and unchanged, the litmus-milk remaining fluid and blue. The growth on all solid media is of a peculiar viscid mucoid character, so much so that it is difficult to make an emulsion of it, the growth on shaking in salt-solution or bouillon can at most be separated into flocculi. This, as the microscope shows, is due to the presence of a viscid, gelatinous, interstitial substance by which the individual yeast-cells are agglutinated.

All cultures, gelatine, agar, sugar-gelatine, sugar-agar, glycerine-agar prove pathogenic when injected subcutaneously or intraperitoneally. Feeding experiments of guinea-pigs made with milk-culture, or with sugar-gelatine or sugar-agar culture remained entirely negative.

The microbe obtained from cultures stains well within its capsule, and except for differences in the size of the spherical cells is morphologically pure yeast; there are at no time found in the cultures those moniliform cylindrical threads which are fairly common in the tissues of the infected guinea-pigs.

We have then here a distinctly pathogenic yeast, belonging to the group of pathogenic blastomycetes to which the researches of Sanfelice, Plimmer and others, in connection with cancer, have drawn attention. From the published reports of these authors, however, our milk yeast in its cultural characters and its pathogenic action on the guinea-pig and rabbit seems distinctly different from those found in cancer.

Conclusions.

To sum up the following are the experimental results of the bacteriological examination of the milk samples and secretions of diseased udders:

(1) 7 p.c. of the samples of "country" milk produced typical true tubercle in the guinea-pig.

(2) 8 p.c. of the samples of "country" milk produced typical pseudo-tuberculosis (non-acid fast bacillus of pseudo-tuberculosis A. Pfeiffer).

(3) 1 p.c. of milk samples produced diphtheria in the guinea-pig, yielding the typical true *B. diphtheriae*.

(4) 1 p.c. of milk samples caused a chronic disease (in most cases with fatal results) due to a pathogenic torula apparently differing in cultural and physiological characteristics from the torula (pathogenic blastomycetes) obtained by Sanfelice, Plimmer and others from human cancer.

(5) Out of the secretions of the cow's udder two pyogenic microbes were obtained: *B. diphtherioides* and *Streptococcus radiatus* (*pyogenes*).

INDUSTRIAL LEAD POISONING.

By T. M. LEGGE, M.D.,

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THE principal data which form the basis of the present paper were tabulated by the writer for the Annual Report of the Chief Inspector of Factories for 1899, and were obtained as a result of an enactment in the Factories and Workshops Act of 1895. This enactment requires every medical practitioner attending on or called in to visit a patient whom he believes to be suffering from lead poisoning, contracted in a factory or workshop, to notify the case forthwith to the Chief Inspector of Factories at the Home Office; and a similar obligation is imposed on the occupier of a factory or workshop to send written notice of every such case to the Certifying Surgeon and Inspector of Factories for the district.

In form there is close similarity between this section and that in the Infectious Diseases (Notification) Act requiring the medical practitioner and householder to notify the medical officer of health of every case of scarlet fever, diphtheria, etc. It is common knowledge that as regards the householder this enactment has been allowed to become a dead letter (although it still remains on the statute) because he is thereby credited with medical knowledge which cannot be expected of him. The case of the occupier of a factory is perhaps not quite on all fours with that of the householder, because, employing persons as he does for his own profit on work in which lead is used, his duty to take every practicable precaution is apparent, and should the injurious effects of lead show themselves in one of the workers his share in the responsibility for this must, within reason, be brought home to him.

As one main object of notification under the Infectious Diseases (Notification) Act is to protect the community against a scourge such as

diphtheria, so repeated notifications of lead poisoning in the same trade may lead to the institution of special regulations for the industry as a whole. As a matter of routine, a notification leads to an inquiry to see whether regulations already in force have been infringed in the particular workplace or not, and as to how far there may have been contributory negligence on the part of the sufferer.

In scarlet fever, in small-pox, etc. the symptoms are, within well-recognised limits, precise; in lead poisoning the differential diagnosis has or ought to be made from a variety of the commonest every-day ailments—headache, anaemia, rheumatism, abdominal pain. In the diseases notifiable under the Infectious Diseases (Notification) Act the patient is almost invariably laid up in bed, whereas in lead poisoning notification is not infrequently made of persons who do not lose a single day's work. There is no standard of what constitutes lead poisoning just as there is no standard of what constitutes ptomaine poisoning, or poisoning by arsenic or mercury. But common sense points to the desirability of fixing in some way the degree of the severity of symptoms which should lead to notification, and there are few medical men probably who would deny that symptoms, the result of the absorption of lead, or arsenic, or mercury, necessitating absence from work, should at any rate constitute sufficient grounds for this.

The notification of the practitioner gives as a rule no indication of symptoms beyond his belief that the case is one of lead poisoning. Details as to these are obtained by examination a day or two later of the patient by the Certifying Surgeon; and the tables which follow are based entirely on an analysis of these reports. In from ten to fifteen per cent. of the cases he is unable to confirm the diagnosis. These cases are marked doubtful, but with a very few exceptions where the evidence against lead poisoning is conclusive are all included in the return. This accounts to some extent for what may be considered the unduly large number of cases in which the severity of the symptoms and the number of the attack appear in the "not stated" column, and the symptoms are recorded as "uncertain." With a malady then showing such extraordinary range in severity as lead poisoning, and attended with such difficulty in diagnosis, a tabulation only of the number reported as so suffering conveys hardly any useful information. It is when the cases are carefully analysed, distinguished according to their severity, to the length of time of exposure to the injurious action of lead, to the number of previous attacks, to the nature of the symptoms, and to the precise occupation that their true value is brought out. If the figures given

are the first of their kind and to be accepted therefore with reservation any doubt attaching to them will be dispersed by the experience of similar figures in future years.

The following table (p. 100) relates to 1130 cases of lead poisoning reported upon by Certifying Surgeons in 1899. It does not include the cases that were notified as occurring among house painters and plumbers for the reason that to these workers the provisions of the Factory Acts do not in general apply, nor does it include a small number of persons notified who for one reason or another could not be traced. The total number of cases of lead poisoning included in the published returns in 1899 as having been contracted in a factory or workshop was 1258.

The reports upon which this table is based describe not only the particular attack but also the general condition of the patient at the time of the attack. Colic is as a rule the symptom which leads to notification, but if on examination old-standing paralysis is found the Certifying Surgeon makes mention of it in his report. This has led in a few cases to anomalies in the column as to "severity," for if the particular attack was slight, even although paralysis was present, the "severity" might be described as slight. It would seem desirable to regard the general condition of the worker rather than the symptoms of the particular attack as the basis of the description "severity" so far as this may be attributable to lead. The personal element of course enters into the report. Symptoms which one certifying surgeon regards as slight, another might consider moderate or even severe. All cases which were marked "fairly severe" or "rather severe" were classed under the heading "severe." Some error may have entered into the description of symptoms as "moderate," for all cases which were reported as "not severe" (obviously they must have been moderate or slight) were classed under that heading.

Very frequently a combination of symptoms is given, and when this is the case each one of them has been entered. The total number of symptoms, therefore, considerably exceeds the number of reported cases, but this does not interfere with the correctness of the estimate of each one as a percentage on the total number reported.

It need hardly be said that the symptoms are usually very shortly described, and the reports therefore are very different from the accurate description of cases to be found in hospital records. They will not admit, for example, where paralysis is mentioned, except rarely, of further tabulation according to the precise group of muscles affected.

A fuller description of the main symptoms could have been given for each one of the industries named. I only give it for the total number of cases reported because I cannot believe that in all cases, as for instance among white lead workers, the symptoms of anaemia, headache, and arthralgia are recorded as often as they must have been present, and I regard therefore the figures given under these heads as too low.

MAIN SYMPTOMS REFERABLE TO LEAD.

	Digestive		Anaemia		Headache		Paretic		Encephalo- pathic		Rheum- atic		Uterine	Uncertain	
	M.	F.	M.	F.	M.	F.	M.	F.	M.	F.	M.	F.	F.	M.	F.
Total	763	119	107	37	59	42	129	13	35	8	58	8	7	64	17
Per cent. of reported cases }	80.0	67.6	11.2	21.0	6.2	23.9	13.5	7.4	3.7	4.5	6.0	4.5	4.0	6.7	9.7

Under smelting are included no less than 28 cases among workers in spelter, where the proportion of lead in the zinc is usually less than 5 per cent. The poisoning is attributable to fumes containing probably oxide and sulphide of lead. In printing, file-cutting, and plumbing, the handling of, or inhalation of dust from, metallic lead is the source of the poisoning. By far the largest number of cases of plumbism are found in the next group of industries, and are attributable in great measure to the inhalation of the dust from the manufacture of carbonate of lead in white lead factories; to the use of white lead in the glazes required in china and earthenware works and enamelling of iron plates; to its use in the putty powder required in glass polishing; and to the grinding of white lead in the manufacture of paints and colours. The inhalation of the dust from the red oxide of lead is responsible for most of the cases in electrical accumulator works. In ship-building also the indispensable qualities which red lead paint possesses of forming a durable coat for iron plates causes it to be much used with as a result a certain amount of lead poisoning.

The table as arranged is capable of showing the amount and degree of poisoning arising from fumes, from handling lead, from salts of lead in the form of dust, and from salts of lead in the form of paint. Bearing in mind as involving a possible fallacy that the figures do not take into account the duration of employment, but include chronic cases as well as first attacks, they certainly seem to show that the slower and more insidious form of lead poisoning brought about by the handling of

ANALYSIS OF REPORTS ON LEAD POISONING

Occupation	Severity of Symptoms									
	Severe		Moderate		Slight		Not stated		Total	
	M.	F.	M.	F.	M.	F.	M.	F.	M.	F.
Smelting { No. of cases ...	9	—	18	—	24	—	1	—	52	—
{ Per cent. ...	17·3	—	34·6	—	46·2	—	2·0	—	—	—
Brass { No. of cases ...	5	—	1	—	4	1	1	—	11	1
{ Per cent. ...	—	—	—	—	—	—	—	—	—	—
Sheet-lead { No. of cases ...	11	—	5	—	10	—	—	—	26	—
{ Per cent. ...	44·3	—	19·2	—	38·5	—	—	—	—	—
Printing { No. of cases ...	7	—	6	1	8	—	4	—	25	1
{ Per cent. ...	28·0	—	24·0	—	32·0	—	16·0	—	—	—
File-cutting { No. of cases ...	21	1	3	—	12	—	3	—	39	1
{ Per cent. ...	53·9	—	7·7	—	30·8	—	7·7	—	—	—
Plumbing { No. of cases ...	7	—	2	—	5	1	2	—	16	1
{ Per cent. ...	—	—	—	—	—	—	—	—	—	—
Tinning and enamelling of iron holloware { No. of cases ...	5	1	2	1	4	2	—	—	11	4
{ Per cent. ...	—	—	—	—	—	—	—	—	—	—
White lead { No. of cases ...	79	3	22	—	232	17	7	2	340	22
{ Per cent. ...	23·2	13·6	6·5	—	68·3	77·3	2·1	9·1	—	—
Red lead { No. of cases ...	6	—	3	1	9	—	1	1	19	2
{ Per cent. ...	—	—	—	—	—	—	—	—	—	—
Earthenware { No. of cases ...	35	22	16	18	62	67	6	4	119	111
{ Per cent. ...	29·4	19·8	13·4	16·2	57·1	60·4	5·0	3·6	—	—
Litho-transfers { No. of cases ...	2	1	—	2	3	1	1	—	6	4
{ Per cent. ...	—	—	—	—	—	—	—	—	—	—
Glass { No. of cases ...	2	1	1	—	4	—	—	—	7	1
{ Per cent. ...	—	—	—	—	—	—	—	—	—	—
Enamelling of iron plates { No. of cases ...	2	1	1	2	3	1	—	—	6	4
{ Per cent. ...	—	—	—	—	—	—	—	—	—	—
Electric accumulators ... { No. of cases ...	8	1	4	—	19	—	—	—	31	1
{ Per cent. ...	—	—	—	—	—	—	—	—	—	—
Paints and colours ... { No. of cases ...	20	—	7	—	16	1	4	—	47	1
{ Per cent. ...	42·5	—	14·9	—	34·0	—	8·5	—	—	—
Coach-painting, &c. ... { No. of cases ...	25	—	9	—	17	—	7	—	58	—
{ Per cent. ...	43·1	—	15·5	—	29·3	—	12·1	—	—	—
Ship-building { No. of cases ...	6	—	8	—	16	—	—	—	30	—
{ Per cent. ...	20·8	—	26·7	—	53·3	—	—	—	—	—
Paint in other industries, excluding house-painters { No. of cases ...	24	1	10	1	11	3	2	—	47	5
{ Per cent. ...	51·1	—	21·3	—	23·4	—	4·3	—	—	—
Other industries { No. of cases ...	22	5	12	5	26	3	4	4	64	17
{ Per cent. ...	—	—	—	—	—	—	—	—	—	—
Total	306	37	130	31	485	96	43	11	954	176
Per cent. of reported cases ...	32·0	21·0	13·6	17·6	50·8	54·5	4·5	6·3	—	—

BY CERTIFYING SURGEONS IN 1899.

Number of Attack								Main Symptoms referable to Lead							
1st		2nd		3rd, or Chronic		Not stated		Digestive		Paretic		Encephalo- pathic		Uncertain	
M.	F.	M.	F.	M.	F.	M.	F.	M.	F.	M.	F.	M.	F.	M.	F.
27	—	13	—	11	—	1	—	42	—	11	—	—	—	1	—
51·9	—	25·0	—	21·2	—	2·0	—	80·8	—	21·2	—	—	—	2·0	—
8	1	1	—	1	—	1	—	7	—	4	—	—	—	1	—
—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
14	—	6	—	4	—	2	—	19	—	7	—	1	—	—	—
53·9	—	23·1	—	15·4	—	7·7	—	73·1	—	26·9	—	3·8	—	7·7	—
15	—	5	1	2	—	3	—	14	—	6	1	2	—	—	—
60·0	—	20·0	—	8·0	—	12·0	—	56·0	—	24·0	—	8·0	—	20·0	—
11	1	9	—	15	—	4	—	21	—	17	—	4	—	2	—
28·2	—	23·1	—	38·5	—	10·3	—	53·9	—	43·6	—	10·3	—	5·1	—
9	1	2	—	4	—	1	—	7	—	3	—	—	—	4	—
—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
5	2	4	1	2	1	—	—	11	—	4	—	—	—	1	—
—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
277	14	38	3	15	2	10	3	292	17	19	1	17	—	13	4
81·5	63·6	11·2	13·6	4·4	9·1	2·9	13·6	85·9	77·3	5·6	—	5·0	—	3·8	—
14	—	4	—	—	—	1	—	17	—	5	—	—	—	1	1
—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
66	84	30	10	20	8	3	9	89	77	11	6	6	7	7	10
55·5	75·7	25·2	9·0	16·8	7·2	2·5	8·1	74·8	69·4	9·2	5·4	5·0	6·3	5·9	9·0
3	2	2	2	—	—	1	—	5	4	—	—	1	—	—	—
—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
4	—	—	—	3	1	—	—	6	—	1	1	—	—	—	—
—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
6	2	—	2	—	—	—	—	6	3	—	—	—	—	—	—
—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
24	1	4	—	2	—	1	—	30	—	2	—	—	—	1	—
—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
29	1	11	—	5	—	2	—	45	1	7	—	2	—	2	—
61·7	—	23·4	—	10·6	—	4·3	—	95·8	—	14·9	—	4·3	—	4·3	—
20	—	5	—	26	—	7	—	43	—	10	—	—	—	11	—
34·5	—	8·6	—	44·8	—	12·1	—	74·2	—	17·2	—	—	—	19·0	—
27	—	2	—	1	—	—	—	28	—	3	—	—	—	—	—
90·0	—	6·7	—	3·3	—	—	—	93·4	—	10·0	—	—	—	—	—
21	3	10	1	14	1	2	—	34	3	10	—	2	—	5	1
44·7	—	21·3	—	29·8	—	4·3	—	72·3	—	21·3	—	4·3	—	10·6	—
13	10	6	4	13	1	3	2	47	12	9	1	—	1	6	1
—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
623	122	152	24	138	14	42	14	763	119	129	13	35	8	62	17
65·3	69·3	16·0	13·6	14·4	7·9	4·4	7·9	80·0	67·6	13·5	7·4	3·7	4·5	6·5	9·7

metallic lead and the absorption of the salts of lead in the form of paint is more severe in the long run than that brought about by salts of lead in the form of dust or from fumes.

Comparing the figures for smelting as representing fumes, the combined totals for sheet lead and printing as representing handling, white lead, and china and earthenware as representing dust, and coach-painting, ship-building, and paint used in other industries as lead salts in the form of paint, it will be seen that the percentage of severe cases is much greater in the handling of lead and in painting.

	Severity of Symptoms				Number of Attacks				Main Symptoms		
	Severe	Mod- erate	Slight	Not stated	1st	2nd	3rd, or chronic	Not stated	Diges- tive	Paretic	Indefi- nite or not stated
Fumes ...	17.3	34.6	46.2	2.0	51.9	25.0	21.3	2.0	80.8	21.2	2.0
Dust ...	24.6	8.4	64.6	2.4	75.4	14.9	7.7	2.9	83.8	6.6	4.4
Metallic lead	34.6	21.2	34.6	7.7	55.8	21.2	11.5	9.6	63.5	25.0	13.5
Paint ...	40.7	20.0	32.6	6.7	50.4	12.6	30.4	6.7	77.8	17.0	11.9

The reports bring out very clearly remarkable differences in the average age of those attacked in one and another industry and the average length of time spent in it—points which are of moment when the question of administrative interference arises. Thus taking three industries, china and earthenware, white lead, and file cutting, the age distribution of the persons attacked and the average duration of employment prior to the particular attack for which they were reported is as follows:—

	Age distribution		Duration of employment	
	Under 30 years	30 and over	Under 5 years	5 and over
China and Earthenware per cent.	59.4	40.6	52.2	47.8
White lead	45.7	54.3	86.8	13.2
File-cutting	22.9	77.1	—	100.0

If the figures for all persons attacked in the china and earthenware industry were examined still more closely and distributed according to sex they would show that men continue to work in lead a much longer time than do women. In that industry, for example, of the total number of females reported in 1898, 69 per cent. were attacked before their thirtieth year and 31 per cent. after; whereas in males the numbers were 46 per cent. and 54 per cent. respectively.

The slowness of the onset of symptoms in the case of file-cutters and of those engaged in painting produces probably in the workers a feeling of indifference to the gradual undermining of the constitution, whereas sharp attacks of colic occurring among white lead and pottery workers cause some of them to seek other employment. This is borne out by the column referring to "number of attack" which shows that in white lead works, in paint and colour works, and in females in china and earthenware there is a far greater number of first attacks than there is in the case of file-cutters and painters. This means a constant influx of new hands in the one case which is absent in the other. The figures as to the duration of employment of those attacked in white lead works (13 per cent. only having worked for 5 years and over) stamp it at once as an industry so fraught with danger that not many will continue to work in it for long. A rule giving power to the Certifying Surgeon to suspend temporarily or permanently from employment any person who he thinks incurs grave risk by continuing in his work can inflict little hardship where the age of the workers is low or in an industry like white lead, where the employment is largely casual, but in file-cutting when 77 per cent. are over 30 years of age to enforce such a rule might be attended with more difficulty.

The figures in the main table and in the subsidiary table on "Main Symptoms" do not contribute much to the solution of the question as to whether women are more susceptible to the effects of lead than men. No doubt the symptoms of headache and anaemia and possibly encephalopathy occur in women more frequently than in men. Saturnine palsy, however, is essentially an affection of the male sex, and the figures support the conclusion that the proportion of severe attacks is greater in males than in females.

Evidence on this point can be obtained in another way. In June 1898 the employment of women in the dangerous processes of the white lead manufacture was prohibited and men took their place. In the six months preceding the change in the district of Newcastle 19 males and 66 females were reported as compared with 82 males and 12 females in the succeeding six months. The number of reported cases of lead poisoning in white lead works in 1899 shows further a regrettable increase (possibly due to increased notification of slight attacks) as compared with previous years. These figures, therefore, do not lead to the conclusion that females are more susceptible to lead than males.

The influence of lead on the child-bearing function is of immense

importance, and the following statistics are valuable as being the result of careful personal inquiry into this subject by Miss Paterson and Miss Deane, two of H.M. Inspectors of Factories¹.

“Out of the 77 married women reported during this period [the year ending March 31st, 1897] 15 have been childless and have had no miscarriages; 8 have had 21 still-born children; 35 have had 90 miscarriages, and of these 15 have had no child born; 36 have had 101 living children, of whom 61 are still alive, the great majority of the 40 who are dead have succumbed to convulsions in infancy.”

Mr J. F. Arlidge, Certifying Surgeon for the district of Stoke, has kindly placed at my disposal some very interesting figures on this aspect of plumbism, the result of inquiries made by him of 239 married women working in lead processes in the china and earthenware industry.

From each woman he has obtained facts as to the number of children born, the number of these who have died, and the number of miscarriages in the two periods, (1) previous to lead employment, and (2) during or after employment in lead. He has also tabulated the ages at death and the cause of death (whenever possible) of the children, the number of years of work in lead, and the precise occupation. The figures admit only of general comparison, for to make this accurate it would be necessary to know the potential child-bearing power at each of the two periods in the case of every woman.

The general facts from his figures may be tabulated as follows:

No. of Women	Previous to lead employment				During or after lead employment			
	Children		Pregnancies		Children		Pregnancies	
	Born	Died	Total	Miscarriages	Born	Died	Total	Miscarriages
239	453	183	487	34	499	182	566	67
	100	40·4	100	7·0	100	36·5	100	11·8

The percentage of children who died to the total number born is greater, and the percentage of miscarriages to the total number of pregnancies considerably less, in the period before lead employment than in the period of lead employment.

Mr Arlidge has distinguished miscarriages from premature births and has limited the term miscarriage to premature expulsion of the ovum at any time up to 6½ months of pregnancy. Among the 183 children who died in the period prior to lead employment are included

¹ Annual Report of the Chief Inspector of Factories for the year 1897, p. 53.

14 premature births (7·6 per cent.) and 11 still births (6·0 per cent.), as compared with 17 premature births (9·3 per cent.) and 21 still births (11·5 per cent.), among the 182 who died in the subsequent period. The percentage proportion of still births in the two periods is, therefore, very similar to that of miscarriages as given in the table. There died of convulsions in the period prior to lead employment 33 children (18·0 per cent.), and in that during or after it 28 (15·3 per cent.)—figures which tend to disprove the view that there is special incidence of convulsions on the children of lead workers. Before commencing work in lead 21 of the women had miscarried—a number which is increased to 34 afterwards. Four miscarried in both periods.

Eleven of the 34 miscarriages recorded in the first period were confined to 3 women, and 20 of the 67 recorded in the second also to 3 women. If these extreme cases are excluded the percentage of miscarriages to the total number of pregnancies is reduced to 5·0 and 8·7 respectively. The *proportion* in the two periods, however, remains practically unaltered.

Among the 239 women there were 71 who had had no children prior to work in lead, and in whom the duration of employment had been 10 years and over. It may be assumed, therefore, for them that the whole of their married life was contemporaneous with work in lead, and that in many of them the full measure of fertility had been reached. These 71 women had 302 children (of whom 114 died), and 38 miscarriages, that is, for every 100 children born 37·7 died and 11·1 of every 100 pregnancies resulted in miscarriage.

The number of miscarriages to pregnancies is variously put at from 1—5 to 1—8 or 10. Whitehead from a series of 2000 hospital cases, *i.e.* wage-earners, puts it at 1—7¹. Miss Paterson's and Miss Deane's figures for the 77 notified cases makes the proportion 1—2·3; Mr J. F. Arlidge's for these 71 women working in lead 1—9, and for the whole 239 women prior to lead employment 1—14.

Disuse of lead has recently been found practicable in the hands of some manufacturers in glazes used for the enamelling of iron plates; a spelter bed has been substituted for the leaden bed formerly used in the manufacture of machine-made files; rouge or oxide of tin has been able to replace putty powder in several branches of the glass-polishing industry; yellow and orange aniline dyes have to a large extent taken the place of the yellow and orange chromates of lead in yarn dyeing. Leadless glazes in china and earthenware are on trial,

¹ See *The Causes and Treatment of Abortion*, by R. R. Rentoul, pp. 2—5.

but in this industry the substitution of an almost insoluble double silicate for the carbonate in the glaze seems likely to effect a very material mitigation of the danger.

The description holds good to-day which Tanquerel des Planches¹ wrote in 1838 of the superiority of white lead paint over any other kind: "(1) elle se mêle facilement à l'huile, (2) elle conserve sa couleur dans cette union, (3) elle s'étend aisément sous le pinceau, (4) elle s'applique exactement sur la surface qu'on veut enduire, (5) elle prête le corps convenable et la faculté de sécher promptement aux autres couleurs, (6) elle n'est altérable ni par l'air ni par l'eau; aussi elle préserve le bois de toute corruption"—and only here and there can other substitutes such as zinc white take its place.

The uses of lead and lead compounds have been so long known and the methods of manufacture have undergone so little change that they continue to be carried on often in the same building which served a like purpose a century ago when the value placed on human health was not what it is to-day. Persons exposed to lead with its anaemia-producing effect want sunlight and air which structural conditions often allow to enter but sparingly.

It is unnecessary to insist on cleanliness or of the ample facilities which should be provided for this on the part of the occupier. Mere cleanliness of the hands, however, is not enough where there is question of lead dust. There must be cleanliness in detail of the nails, of the teeth, and of the clothing. And to meet these by the provision of nailbrushes, overalls, and head coverings is not enough. There should be in addition a periodical medical examination at stated times, with power to the appointed surgeon to suspend temporarily or permanently from work those who he thinks are specially susceptible.

The following extract on this point from the suggestions² to such surgeons as have been appointed for this work in white lead factories and china and earthenware works may prove useful:

The necessity for notification does not arise until lead poisoning is diagnosed, and, in general, not unless the symptoms are of such a nature as to require abstention from work, but in deciding the question of suspension or fitness for work in lead other considerations come in. Liability to injurious effects may be indicated by present or past attacks of lead poisoning, by tendency to epilepsy, by marked anaemia not necessarily due to lead, by careless personal habits, such as want of attention to cleanliness of hands and teeth or of clothing, or biting of nails,

¹ *Traité des Maladies Saturnines*, Paris, 1839, Vol. I. p. 123.

² *Memorandum on Industrial Lead Poisoning*, Home Office, 1898.

or taking food in prohibited places, or neglect of other precautions prescribed by Special Rules. Indirectly, shortsightedness predisposes to danger, by causing undue proximity to the work. In women the question of pregnancy has to be borne in mind.

Where the diagnosis is clearly established there should be no hesitation in ordering suspension. Whether the predisposing cause in such instances be natural susceptibility or merely careless habits, the unfitness for work in lead is equally obvious.

Occasionally a case will present itself to the Certifying Surgeon, at the monthly or weekly examination, in which the symptoms are so marked as to warrant notification as well as suspension from work. Such cases should obviously be distinguished from those in which the symptoms are so pronounced that medical attendance is sought by the patient at his home or at hospital, and a note should accordingly be made on the report form.

Short of suspension, conditions are met with which call for a caution to the workers, and careful observation month by month on the part of the Certifying Surgeon. In like manner special attention should be directed at the monthly examination to those who have been allowed to resume work in lead processes after an attack of plumbism, to those who present equivocal symptoms in any way suggesting plumbism, to those who have a marked blue line, and to women workers who are pregnant.

The value of the periodic examination in impressing upon the workpeople the importance of habits of cleanliness, and of attention to the prescribed rules, cannot be too strongly insisted upon, and it would be well if the Certifying Surgeon, in reporting on notified cases, were, as a matter of routine, to note whether from his observation of the home surroundings and general appearance of the patients, as well as from their replies, he is able to draw any conclusion as to their exercise of reasonable care.

The duration of suspension from work rests with the discretion of the Certifying Surgeon. In general, it may be said that a temporary suspension should suffice in cases of slight and transient attack, but repeated or grave attacks call for prolonged abstention from work in lead, and usually for discontinuing it altogether.

It is illegal under section 17, 1891, for a woman to be employed in any factory or workshop within four weeks after confinement; and where the work is such as to involve the grave additional risk of plumbism a longer interval becomes necessary.

This periodical medical examination should not be lightly undertaken. It places grave responsibilities on the surgeon, and its effect for good will depend entirely upon his appreciation of them. In the out-patient room of a hospital a patient comes to tell his symptoms—at a factory the condition has to be judged of sometimes in the face of deliberate misstatements. Hence mere questioning will fail to secure the full benefit from the provision unless every other means for the diagnosis of incipient plumbism is called in aid, such as examination of the condition of the teeth and gums, of the pupils and muscles of the eye, of the colour of the conjunctiva, of the presence or

not of tremor or hysteria, and of the resistance of the extensors of the wrist to forcible flexion.

The question of the removal of lead dust by mechanical means and the practicability of respirators as a means of protection against it opens up a field too wide to enter on here, suffice it to say that while reliance has to be placed less and less on the use of respirators, every day sees improved conditions of work brought about by the introduction of fans so arranged as to catch the dust at the point where it is produced.

A RAPID METHOD OF DETERMINING CARBONIC ACID IN AIR.

(One Figure in the Text.)

By JOHN HALDANE, M.D., F.R.S.

(*From the Physiological Laboratory, Oxford.*)

IN almost all investigations relating to ventilation of inhabited buildings determinations of the carbonic acid present in the air are essential. The method in common use is that of Pettenkofer, or some modification of it. This method has, however, the disadvantage that the determinations necessitate the carrying to and from the laboratory of large bottles, and take a considerable time. To overcome these drawbacks several methods capable of being conveniently applied on the spot have been devised, but their accuracy is hardly sufficient for practical purposes, hence they have not come into general use.

The method now to be described was designed in connection with the work of the Home Office Committee on Ventilation of Factories and Workshops, and the apparatus, the construction of which is shown in the figure, is a simplified form of a gas-analysis apparatus which I described in 1898¹. The practical advantage of the method is that the apparatus can easily be carried about, and is always ready for

¹ *Journ. of Physiol.*, Vol. xxii. p. 465, 1898.

The more important measurements connected with the present apparatus are as follows :—

Internal measurement of case $8 \times 13 \times 3$ inches.

„ „ „, water-jacket $2\frac{5}{8} \times 1\frac{1}{2} \times 7$ inches.

Diameter of bulbs about $1\frac{1}{2}$ inches.

Length of graduated part of burette about 4 inches or 100 mm.

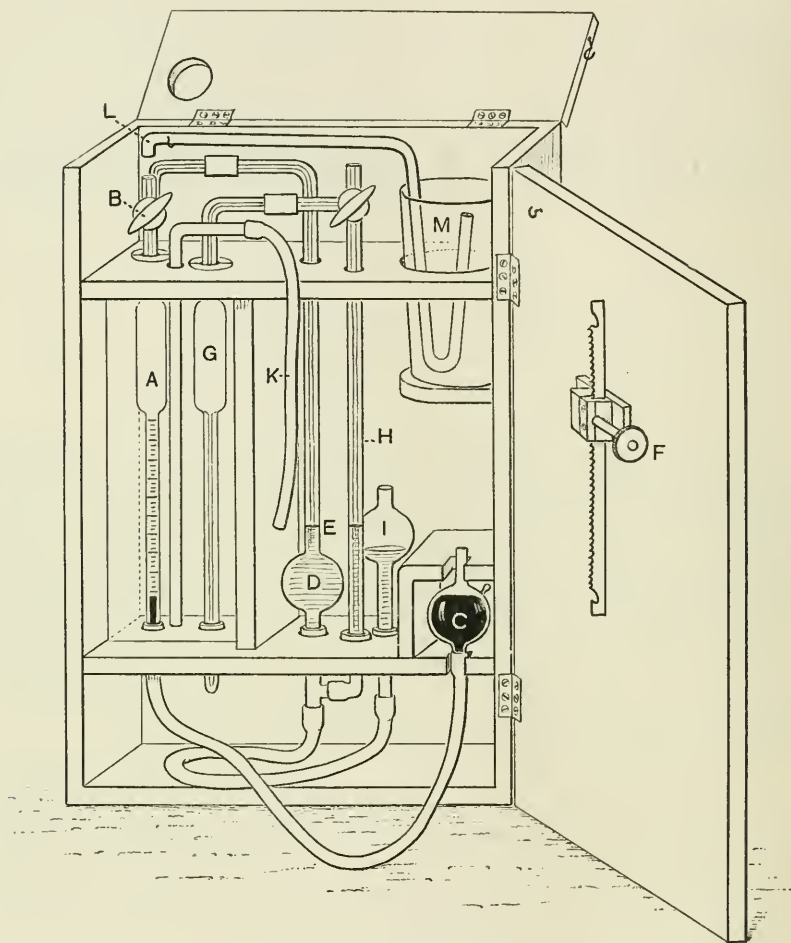
Thickness of wood used throughout = $\frac{3}{8}$ inch.

Weight, including mercury and water, about 6 pounds.

The apparatus has been made for me by Messrs C. E. Muller and Co., 148 High Holborn, London.

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immediate use: that an accurate result can be obtained within about five minutes; and that no calculations are involved.



One-fourth actual size.

The gas burette *A*, which is enclosed in a water-jacket, consists of a wide ungraduated, and a very narrow graduated portion. It holds about 20 c.c. from the tap to the bottom of the scale. The graduated part, which is about 4 inches long, is divided into 100 divisions, each of which corresponds to $\frac{1}{10000}$ th part of the capacity of the burette. The lowest division is marked 0, and the highest 100. Any difference between a reading at or near zero and a second reading is thus shown by the scale in volumes per 10,000.

In using the apparatus the air is first expelled from the gas-burette by opening the three-way tap *B* to the outside, and raising the mercury bulb *C*. The tap is then closed, and the mercury bulb replaced in its stand. On opening the three-way tap again a sample of the air is drawn in, and the level of the mercury falls to near the zero mark. The tap is now opened towards the absorption pipette *D*, which is filled up to a mark at *E* with potash solution, and the sample measured with the precautions to be described below. It is then passed over into the absorption pipette, driven backwards and forwards for a few seconds, and then again measured after the absorption of the carbonic acid. The difference between the two readings gives directly the number of volumes of carbonic acid per 10,000 in the sample of air.

It is evident that the correctness of the analysis depends entirely on the avoidance of errors of various kinds in the two determinations of the volume of the enclosed air. Mistakes might be caused by slight variations in the temperature of the water, or the pressure under which the sample is measured, or in the degree of saturation with moisture of the sample. A variation of 0.1° C. in the temperature of the water in the jacket would, for instance, unless corrected, cause an error of fully \pm volumes per 10,000 in the analysis.

In order to have a sharp index of the pressure under which the air is measured the level, not of the mercury, but of the potash solution in the narrow bore tubing of the absorption pipette, is taken as the index of pressure. At the first measurement the level is accurately adjusted to the mark at *E* by raising or lowering the mercury by means of the rack and pinion arrangement *F*. At the second reading the potash level is again adjusted in the same way. As the potash has a specific gravity of only about a tenth of that of mercury its level is a very delicate index of the pressure. A difference of $\frac{1}{10000}$ th part in the pressure would correspond to a difference of 1 mm. in the pressure of the potash solution, which would be very evident to the eye.

To correct for variations in temperature of the water-jacket a control tube *G* is employed, of a size and shape approximately the same as the gas-burette. The control tube communicates with the potash through the narrow bore glass tube *H*, and before the first measurement is made the level of the potash in *H* is adjusted to the mark by lowering or raising the reservoir *I*, which slides up and down in a loosely fitting cork. At the second measurement the same precaution is taken, so that the air in the control tube occupies exactly the same volume as at the first measurement. As an alteration of

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temperature or of barometric pressure would affect the pressure to an equal extent in the gas-burette and control tube it is evident that the adjustment of the level of the potash reservoir compensates exactly any error which the alteration of temperature or of barometric pressure would cause in the reading of the gas-burette¹.

Before the adjustments of the potash levels are made the water in the jacket is thoroughly mixed by blowing air through it by means of the tube *K*. This manipulation is essential to an accurate result. The tubes *E* and *H* have a bore of about 2 mm. If a narrower bore be employed error may arise through the potash not returning sharply to a perfectly definite level when disturbed.

In order to obviate error due to variations in the saturation of the air both the burette and the control tube are left with a little visible moisture inside. If the burette has once been wetted inside, and as much as possible of the water expelled by raising the mercury, it remains moist for a very large number of analyses, but a little moisture should always be visible. If by any mishap potash should be sucked over into the burette, it, and its connections, must be washed out with dilute acid introduced by the tap.

The accuracy of the graduation can be tested by taking out the burette, filling it with mercury, and weighing what flows out between different points in the graduation. A detached column of mercury should occupy the same number of divisions at all parts of the graduated tube of the burette. The efficient working of the apparatus can easily be ascertained by making an analysis of pure outside air, which should give about 3 volumes per 10,000 (except in dull or foggy weather in towns, where the amount may be a good deal higher.) A further test is to leave a sample of air in the apparatus after the carbonic acid has been absorbed, and see that its volume is not altered after the potash levels have been readjusted for alteration of temperature and atmospheric pressure, or after it has been passed over into the potash pipette, as in an analysis. Any error due to leakage in the

¹ The use of a control tube in gas analysis was first described by Williamson and Russell (*Journal of the Chemical Society*, 1868, p. 238) and afterwards applied in a greatly improved form by Pettersson (*Zeitschr. für analyt. Chemie*, Vol. 25, pp. 467, 479.) The latter along with Palmqvist has successfully applied the same principle to the determination of CO_2 in the air of rooms, and devised for the purpose a special form of gas-analysis apparatus with a narrowed burette similar to that described above, which gives excellent results (*Berichte der deutschen chemischen Gesellschaft*, Vol. 20, p. 2129, 1887).

connections, or blocking of any of them by a drop of liquid would thus be at once revealed.

At the end of an analysis the taps must be turned so as to close the communications between the potash and the burette and control tube: otherwise potash may be sucked in if there is any great fall of temperature or rise of barometric pressure.

The apparatus is so arranged that it can be used either for taking and analysing on the spot examples of air, or for analysing at some convenient place samples which have been collected in small bottles of about 1 oz. capacity. When the former method is preferred a short piece of capillary tubing, which projects through the hole in the cover of the case, is fixed by rubber-tubing on the upper opening of the burette. The burette is filled with mercury, the tap closed, and the mercury reservoir replaced in its stand. The apparatus is then held at the place where the sample is to be taken, and the tap opened, so that the sample is drawn in. During this process the breath should be held so as to avoid any risk of contaminating the sample with expired air. The tap is then turned, and the analysis completed in the ordinary way.

When samples are collected in bottles the latter should be thoroughly clean, and provided with a good cork which has been coated with a thin layer of paraffin wax well melted on. The sample is collected by placing the end of a piece of rubber-tubing in the bottle, and taking in a full breath through the tubing. The bottle is at once firmly corked. For an analysis the piece of bent glass tubing shown at *L* is attached to the opening of the burette and water poured into the glass vessel *M*. The tubing is then filled with mercury by raising the mercury reservoir, the tap closed, and the reservoir replaced. The cork of the bottle is now loosened, the neck immersed in the water, and the cork pushed out under water with the thumb. The bottle is then placed so that the glass tubing projects into it, and the tap opened, so that a sample of the air is withdrawn into the burette. Before turning the tap the bottle is held up, so that the water levels inside and outside of it are equal, and the sample withdrawn is therefore at atmospheric pressure. A second sample cannot be taken from the same bottle, as the presence of the water rapidly affects the percentage of carbonic acid in the air of the bottle.

The following examples give an idea of the degree of accuracy attainable by this method:

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I. Analyses of four bottles full (each bottle held about 30 c.c., or 1 cubic oz.) of air collected in rapid succession in a closed room with gas burning.

Vols. per 10,000.

Bottle A.	15.0	Analysis began 5 minutes after collection.			
Bottle B.	14.8	"	"	10	" " "
Bottle C.	16.2	"	"	20 hours	" "
Bottle D.	15.2	"	"	20 hours	" "

II. Analyses of three bottles of pure outside air, collected simultaneously.

Vols. per 10,000.

Bottle A.	2.8,	Bottle B.	3.6,	Bottle C.	3.2.
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III. Six successive analyses of outside air (night).

Vols. per 10,000.

(1) 3.0,	(2) 3.5,	(3) 3.6,	(4) 3.0,	(5) 3.6,	(6) 3.1.
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These results show that the method is reliable to about 0.5 vols. per 10,000; and this degree of accuracy abundantly suffices for almost all practical purposes.

The manipulations required during an analysis may be recapitulated as follows: (1) Open the tap of the control tube to the air for a moment, and then turn it so as to connect the control tube and potash pressure gauge. (2) Turn the tap of the burette so as to connect the burette and the potash pipette. (3) See that the level of the potash alters sharply and about equally in the tubes when the potash reservoir is raised. (4) Blow air through the water-jacket. (5) Raise or lower the potash reservoir till the potash is exactly at the mark in tube *H*. (6) Raise or lower the mercury reservoir by means of the rack and pinion till the potash in *E* is exactly at the mark. (7) Read off the mercury level on the scale of the burette to .2 of a division. (8) Raise the mercury to the upper hook, so as to drive the air into the potash bulb, then lower it a little and raise it again twice so as to wash any carbonic acid in the connecting tubing into the potash bulb. (9) Return the air to the burette. (10) Blow air through the water-jacket. (11) Adjust the two potash levels as before and read off the mercury level. The first reading subtracted from the second gives the result in volumes per 10,000. (12) Close the two taps.

THE RED COLOUR OF SALTED MEAT.

(One Figure in the Text.)

By JOHN HALDANE, M.D., F.R.S.

(*From the Physiological Laboratory, Oxford.*)

WHEN fresh meat, or blood, is boiled the colour changes from the red of oxyhaemoglobin to a dull, brownish colour. The change of colour is due to splitting up of oxyhaemoglobin into coagulated proteid and haematin, which are precipitated. The dark colour of haematin, mixed with the white of coagulated proteid, gives the dull brown. When, however, meat has been salted, it has a characteristic red colour when cooked. It is evident, therefore, either that ordinary haematin is not split off from the oxyhaemoglobin, or that the colour of the haematin is masked by the presence of another pigment.

The red colour is well known to be caused in some way by the nitre which is always mixed with the salt used in preserving the meat. It has further been recently shown by Kiskalt¹ that meat becomes red when boiled in water containing nitrite, and he suggests that the red colour of salted meat may be due to the presence of nitrite; the manner in which the change of colour is produced has not hitherto been explained, and the experiments now to be described were undertaken with a view to elucidating the matter.

Endeavours were first made to extract the red pigment from cooked salted meat. The red colour was altogether insoluble in water, but a weak solution was obtained by kneading the meat with alcohol. The red colour faded after a time. On spectroscopic examination an ill-defined absorption band covering the *D* line of the solar spectrum could be distinguished, along with strong absorption of the whole of the blue end of the spectrum. The same appearance was seen on cutting a very

¹ *Archiv für Hygiene*, vol. 35, p. 11, 1899.

thin slice of the meat and holding it before the slit of the spectroscope. This spectrum did not seem to correspond to that of any known derivative of haemoglobin, and I was therefore led to examine uncooked salted meat.

On cutting into pieces of uncooked salted meat, obtained from the butcher, I noticed that the colour of the exposed surface was bright red wherever the salt had penetrated, but slowly became dull on exposure to the air. On the other hand, if the salt had not thoroughly penetrated to the centre of the piece the exposed surface had at first the dull bluish tint of reduced haemoglobin, which soon changed into the red of oxyhaemoglobin on exposure to the air. That this red was really due to oxyhaemoglobin was proved by spectroscopic examination. If the part of the meat which had changed from blue to red was covered with a glass plate so as to exclude air it again became blue from reduction of the oxyhaemoglobin, whereas if the part which had become dull on exposure to air was covered it regained its red colour. It was thus evident that some different pigment from ordinary haemoglobin was present in the parts to which the salt had penetrated.

On extracting the freshly exposed salted part with water the red pigment was found to be quite soluble, and to give a spectrum not altered by warming the solution with ammonium sulphide, and possessing two absorption bands at about the position of the oxyhaemoglobin bands, but not nearly so well defined. This spectrum was found to be identical with that of nitric oxide haemoglobin (no. 1 of figure); and the behaviour of the pigment in all other respects showed that it was nothing else but pure NO-haemoglobin.

This substance, which was discovered by Hermann¹ in 1865, has hitherto not attracted very much attention, as it was supposed only to occur as a laboratory product. It is prepared by passing nitric oxide through blood which has been deprived of oxygen, care being at the same time taken to exclude from the blood the nitric peroxide formed by the nitric oxide as it escapes into the air. The spectrum of NO-haemoglobin was described in general terms by Hermann. As, however, no figure showing the appearance and exact position of the bands has hitherto been published the accompanying sketch will be of service. For comparison the spectra of oxyhaemoglobin and CO-haemoglobin solutions of equal concentrations are also given. It will be seen that in the case of NO-haemoglobin both bands are less well-defined, and that the band in the neighbourhood of the *D* line extends slightly but quite distinctly

¹ *Archiv für Anat. und Physiol.*, p. 469, 1865.

over the *D* line towards the red. The appearance and position of this band at once distinguish NO-haemoglobin from either oxyhaemoglobin or CO-haemoglobin.

C	D	E	<i>b</i>	F	
					1. Nitric oxide haemoglobin.
					2. Oxyhaemoglobin.
					3. Carbonic oxide haemoglobin.
					4. Nitric oxide haemochromogen.
					5. Obtained by action of nitrous acid on haematin.

Undiluted blood has the same florid red colour whether it be saturated with oxygen, carbonic oxide, or nitric oxide, but in a thin layer of blood, or a dilute solution, NO-haemoglobin is slightly more pink, and less yellow, than oxyhaemoglobin, and much less pink than CO-haemoglobin. Solutions of oxyhaemoglobin and CO-haemoglobin may also be distinguished by the fact that on adding ferricyanide and acidifying slightly with acetic acid they at once give the spectrum and colour of pure methaemoglobin, whereas a solution of NO-haemoglobin is only slowly and partially decomposed, the two bands of NO-haemoglobin remaining still visible along with the methaemoglobin band in the red, and the colour of the solution being brownish-red. On exposure to air NO-haemoglobin is slowly converted into methaemoglobin.

On boiling, NO-haemoglobin will be found to give a red coagulum. This fact alone is sufficient to distinguish it from CO-haemoglobin or oxyhaemoglobin. Reduced haemoglobin also gives a red coagulum due to the formation of haemochromogen ("reduced haematin"), but the colour at once changes to the ordinary dull brown or grey on exposure to the air. The formation by NO-haemoglobin of a permanent red colour on boiling of course accounts for the red colour of cooked salt meat.

A solution of the red pigment formed on boiling may be obtained by slightly diluting and adding alkali or acetic acid to the blood, so that it does not coagulate. The colour of the solution is ruby-red. On dilution it appears brownish-red. On spectroscopic examination of the diluted solution, whether alkaline or acid, the spectrum shown at

no. 4 of the figure is seen. There is a not very sharply defined absorption band covering the *D* line, and with a centre somewhat to the blue side of the latter: at the same time the whole of the blue end of the spectrum beyond this band is more absorbed than the red end, and a second very faintly defined band can just be distinguished near *E*, in the position shown.

It is natural to suppose that the substance in solution is a nitric oxide compound of haematin, or rather of haemochromogen. To test this supposition nitric oxide was passed through solutions in water containing ammonia of haematin prepared both by MacMunn's method¹ and by simply boiling blood with caustic soda. The colour of the solutions soon changed to red, and the spectrum described above appeared. The spectrum of haemochromogen was, however, sometimes at first visible, and the action of the nitric oxide was, evidently, first to reduce the haematin to haemochromogen, and then to combine with the latter.

Nitric oxide haemochromogen has already been described by Linossier², who obtained it by passing nitric oxide through haematin solutions. He described its spectrum as containing two bands between *D* and *E*, and similar to those of oxyhaemoglobin, but with the second band much less sharply defined than the first. This description does not correspond well with that given above, and I was at first much puzzled by the want of agreement. I obtained, however, a spectrum which seems to correspond somewhat better to his description by prolonged bubbling of nitric oxide through slightly alkaline solutions of haematin. This spectrum is shown at no. 5 in the figure. There are two well-defined absorption bands, of which one is near, but quite clear of, the *D* line on the blue side, and the second, which is more diffuse, extends on both sides of *E*, reaching nearly to *b*. The colour of the solution was red. On testing its reaction I found that it had become strongly acid, and unless the reaction became acid the spectrum was not obtained. On the other hand I could not obtain it by acidifying, even with mineral acids, solutions of NO-haemochromogen prepared by boiling NO-haemoglobin with alkali. This spectrum is thus not simply that of an acid solution of ordinary NO-haemochromogen. Finally, I found that on adding nitrite to a solution of NO-haemochromogen or ordinary haematin, and then acidifying strongly, the spectrum was at once obtained. Its appearance on prolonged passage of nitric oxide

¹ *Journ. of Physiol.* vol. 6, p. 22, 1885.

² *Comptes Rendus*, vol. 104, p. 1296, 1887.

through haematin is thus apparently due to the presence of free nitrous acid formed in the test-tube by the action of air. Further investigation must decide the nature of the compound in question.

The origin of the NO-haemoglobin present in uncooked salt meat remains to be discussed. A watery extract of the latter, or a sample of the brine in which meat has been salted, gives with potassium iodide, sulphuric acid, and starch solution a very strong blue coloration, indicative of the presence of nitrite, and that the presence of nitrite in some way causes a red colour was, as before mentioned, proved by Kisskalt. The action of nitrites on blood was first observed and investigated by Gamgee¹, who found that the blood assumes a chocolate colour, and completely loses its characteristic power of taking up oxygen and yielding it again to a vacuum. He also described the alteration in spectrum, which was generally assumed until lately to be due simply to the formation of methaemoglobin from the oxyhaemoglobin of the blood. It was, however, pointed out recently by Makgill, Mavrogordato, and myself² that the spectrum and colour of oxyhaemoglobin acted on by a nitrite are not those of pure methaemoglobin, but of a mixture with the latter of about a fourth as much of NO-haemoglobin³.

On further investigation of the action of nitrites on blood I have found that if the blood be deprived of its oxygen previously to the action of nitrite an almost pure spectrum of NO-haemoglobin is obtained, only a little methaemoglobin being formed. To carry out the experiment some fresh blood slightly diluted was introduced into a glass vessel fitted with a stop-cock at both ends. The vessel was connected through one of the taps with an ordinary filter-pump, slightly warmed, and all the oxygen boiled out. The tap was then turned off, and a few drops of a saturated solution of sodium nitrite were allowed to be sucked in, without any air. After a short time the spectrum of reduced haemoglobin at first seen in the blood changed into that of nitric oxide haemoglobin, accompanied by a weak methaemoglobin band in the red. The latter band could only be seen in concentrated solutions.

Nitrites not only act on oxyhaemoglobin and reduced haemoglobin,

¹ *Phil. Trans.* p. 589, 1868.

² *Journ. of Physiol.* vol. 21, p. 165, 1897.

³ The difference in colour and spectrum between pure methaemoglobin, as obtained for instance by adding ferricyanide to diluted blood, and the mixture of pigments obtained with a nitrite is rendered very evident if the solutions be slightly acidified by adding a drop of acetic acid, or simply by blowing expired air through them. The nitrite product has a more or less red colour, and shows the two bands of NO-haemoglobin in addition to the spectrum of acid methaemoglobin, while the other solution has no trace of red in it.

but also on methaemoglobin. If nitrite is added to methaemoglobin prepared from blood with ferrieyanide the colour alters to the same reddish-brown tint as is obtained when nitrite acts on oxyhaemoglobin, and the same spectrum is seen, corresponding to a mixture of NO-haemoglobin and methaemoglobin. The explanation of the peculiar behaviour of nitrites towards oxyhaemoglobin, reduced haemoglobin, and methaemoglobin is probably to be found in the facts that (1) haemoglobin readily combines either with nitric oxide to form NO-haemoglobin or with nascent oxygen to form methaemoglobin: (2) nitrous acid easily yields up simultaneously nitric oxide and oxygen: (3) nitric oxide readily combines with either oxygen or haemoglobin. If no extraneous oxygen is present, as when reduced haemoglobin is acted on by the nitrite, both the nitric oxide and the oxygen yielded up by the nitrite will combine with the haemoglobin; and as far more nitric oxide than oxygen is yielded up, more NO-haemoglobin than methaemoglobin will be formed. On the other hand, when oxyhaemoglobin or methaemoglobin is acted on the nitric oxide will be free either to combine with haemoglobin or with the extra oxygen available; and a balance will be struck depending on the relative strengths of the various affinities.

NO-haemoglobin may be formed by the action of nitrite in a less direct manner than that described above. In the paper just referred to we noted the fact that when the body of an animal poisoned by nitrite is left for a day or two the methaemoglobin which was present at death disappears, and the haemoglobin is completely converted into NO-haemoglobin¹. This observation is explained by the facts adduced in the preceding paragraphs. Bacteria and possibly other agents reduce the methaemoglobin, and the nitrite present then converts the reduced haemoglobin into NO-haemoglobin. The same change occurs in salted meat, and accounts for the fact that the red colour of the NO-haemoglobin, where it has faded in consequence of the production of methaemoglobin by the action of the air, may be restored by leaving the surface covered up so as to exclude air. In this process bacteria doubtless bring about the reduction, as their growth is only hindered, and not prevented by the action of the salt. Meat left in the brine used for salting was found to become putrid after some weeks.

When oxygenated blood to which nitrite has been added is boiled the coagulum obtained is reddish-brown in consequence of the presence in the clot of NO-haemochromogen produced by decomposition of the NO-haemoglobin formed along with methaemoglobin by the nitrite. If

¹ *Journ. of Physiol.* vol. 21, p. 168, 1897.

the boiling be continued the coagulum becomes redder, and similar in tint to the coagulum from blood saturated with NO. Reducing substances present in the blood appear to favour the further production of NO-haemochromogen. If the blood has first been boiled and the nitrite is then added to the clot the coagulum does not become red.

Nitrites do not act on alkaline solutions of haematin, but if a slightly alkaline haematin solution be reduced with ammonium sulphide in presence of nitrite the haemochromogen spectrum at first seen is replaced entirely by that of NO-haemochromogen. The presence of excess of alkali prevents this change. When reduced haemoglobin is boiled there is formed haemochromogen instead of haematin; and the haemochromogen is of course capable of forming NO-haemochromogen with any nitrite present. It must also be remembered that the reducing substances present in blood or muscle may absorb oxygen energetically when heat is applied, and that in this way even oxyhaemoglobin may yield haemochromogen and be converted by nitrite into NO-haemochromogen. These facts explain the very familiar observation that when a pie is made from fresh meat along with a little bacon, or other salted meat, the fresh meat is red when the pie is cooked.

The nitrite found in salted meat is probably partly formed by the reducing action of bacteria on the nitre which is always mixed with the salt. The action of bacteria is, however, not necessary, as it has recently been shown by Abelous and Gérard¹ that the fresh tissues themselves contain a substance capable of reducing nitrates to nitrites, and that this substance acts even in the presence of antiseptics.

Nitrites are somewhat actively poisonous; and as has been recently shown² they cause death by asphyxia through their action on the haemoglobin and the consequent interference with the oxygen supply to the tissues. Their presence in very appreciable quantity in raw salted meat might thus seem to be possibly harmful. On testing cooked salt meat, or the water in which it has been boiled, I have only sometimes found nitrite, however; and it is well known that nitrites are destroyed by prolonged heating. In some samples of ordinary well-preserved tinned meat I have also found no nitrite, although it is probable that the nitrate present would be reduced to nitrite if the sterilisation of the meat were incomplete.

Nitrates are a good deal less poisonous than nitrites, but their presence in salted meat when this is regularly used as food is probably

¹ *Comptes Rendus*, vol. 129, pp. 56 and 124, 1899.

² Haldane, Makgill, and Mavrogordato, *Journ. of Physiol.* vol. 21, p. 160, 1897.

not a matter of indifference. In a future paper I hope to give the results of further investigations on this subject.

Chief Conclusions.

1. The red colour of cooked salt meat is due to the presence of NO-haemochromogen.

2. The NO-haemochromogen is produced by the decomposition by heat of NO-haemoglobin, to which the red colour of unsalted meat is due.

3. The NO-haemoglobin is formed by the action of nitrite on haemoglobin in the absence of oxygen, and in presence of reducing agents.

4. The nitrite is formed by reduction within the raw meat of the nitre used in salting.

5. The nitrite is destroyed by prolonged cooking.

AN EXPERIMENT ON THE EFFECT OF INHALATION OF ETHYLENE.

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THE opinion has recently been expressed¹ that the poisonous action of ordinary coal-gas and carburetted water-gas is probably in part due to the "Illuminant" hydro-carbons, of which ethylene is the chief, and not simply to carbonic oxide. In consequence of the doubt existing on this point one of us was asked by the recent Departmental Committee of the Home Office on Water-Gas to investigate the matter, and the results of the experiments which we were then able to make appeared in the Committee's Report². In the main series of observations the animal was placed in a respiration chamber through which a current of air was passing at a known rate. With the current, before it entered the chamber, a known percentage of coal-gas or carburetted water-gas was mixed. It was found that whether ordinary coal-gas or carburetted water-gas was used the symptoms observed were those of carbonic oxide poisoning, and corresponded exactly to the percentages of carbonic oxide present. We also found that Benzene, which is one of the "illuminants," is present in proportions far too small to contribute to the toxic effects of coal-gas or carburetted water-gas. Finally, we endeavoured to investigate separately the action of ethylene. The ethylene we then used was prepared in the ordinary way from sulphuric acid and alcohol, and when about 10% of the gas was mixed with air and supplied to an animal very distinct toxic symptoms were produced.

¹ See Sir Henry Roscoe's evidence, Appendix to the Report of the Water-Gas Committee (Parliamentary Paper), p. 109, 1899.

² Appendix, p. 127.

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On shaking the ethylene with blood solution we found, however, that carbonic oxide was present; and a determination of the latter by the blood method¹ gave 1·8%. The carbonic oxide present was thus sufficient to account for the symptoms observed.

The experiment now to be described was made with ethylene prepared from ethylene dibromide by the action of a zinc-copper couple². The sample of gas obtained was found, when tested with blood solution, to be free from carbonic oxide, and gave, on analysis, the following result:

Ethylene	96·0
Oxygen	0·5
Nitrogen	1·7
Hydrogen	1·4
Ethane	0·4
	<hr/> 100·0

We filled a cylinder of 1100 c.c. capacity with a mixture of 72·5% of the ethylene and 27·5% of oxygen. After thorough mixture of the gas a mouse was placed in the cylinder. At the end of a minute the mouse seemed to have some difficulty in walking, as if slight intoxication had resulted from breathing the ethylene. Careful observation was continued for an hour, but the symptoms did not become in any way more marked. The mouse was then taken from the cylinder, and ran along the floor at once and with perfect ease.

From this experiment it is evident that the effects produced by ethylene are very slight, even when 72% of the gas is present. Ordinary coal-gas does not contain more than 3 or 4% of ethylene, and in air which has been rendered just poisonous by the addition of coal-gas only about 0·2% of ethylene would be present, so that it is impossible to attribute to ethylene any part of the poisonous action of coal-gas.

¹ Haldane, (1898) *Journal of Physiol.*, vol. 22, p. 478.

² Roscoe and Schorlemmer's *Chemistry*, Vol. III. pt. 2, p. 37 (1890).

ARTIFICIAL MODIFICATIONS OF TOXINES WITH SPECIAL REFERENCE TO IMMUNITY.

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WHATEVER may be the final form of the theories of Ehrlich as to the constitution of the bacterial toxins and as to the essential nature of immunity there can be no doubt of the stimulus they have given and are likely to give to inquiries relating to these subjects. As the present paper is intended to give a preliminary account of some researches bearing on the reactions of certain toxins it may be useful to recall the main features of the theories in question so far as they relate to the toxins investigated. The latter were all members of the group, of which the poisons formed by the bacillus of diphtheria are usually taken as the type, and it will be remembered that it was these diphtheria toxins on the investigation of which Ehrlich first based his theories. His fundamental experiments were, briefly, as follows. In default of obtaining the poisons in a pure form and weighing them, the standard amount of diphtheria toxin to which all other quantities are referred is the amount of any impure sample which contains sufficient to kill a guinea-pig of 250 grammes in four days. This is the minimal lethal dose ("M.L.D."). Ehrlich taking one unit of an anti-diphtheritic serum which he made his *standard* serum found how much of any particular toxin could be mixed with this unit *in vitro* so that when the mixture was injected into a guinea-pig no illness took place. Now supposing we had such a mixture of a simple poison like strychnine with its antidote, if such a thing existed, and if we added to this mixture one M.L.D. of strychnine and injected the whole into an animal we would expect it to die. Ehrlich found that on performing

precisely such an experiment with diphtheria toxine and antitoxine the animal did not die, in fact, in the case of the specimens of toxine investigated by him, before death would take place, there had to be added to the neutral mixture of toxine and antitoxine as much of the toxine as would by itself have killed many animals. The explanation which he gives of this phenomenon is that the crude toxine is not a simple chemical body but a mixture containing a series of bodies which on the one hand have different degrees of affinity for the antitoxine and on the other have different degrees of toxicity for an animal. Thus if the antitoxine in the original mixture were partly saturated by bodies which had great affinity for it and also great toxicity and partly by bodies which had less affinity and also less toxicity, on fresh toxine being added to such a mixture the less avid, less toxic bodies would be displaced by part of the fresh very avid, very toxic bodies. The last would however be prevented having a toxic action on being injected into an animal by being already saturated by the antitoxine. The free toxine added to the neutral mixture would thus after this rearrangement of its constituents had taken place be robbed of part of its toxic power and an amount greater than a M.L.D. would have to be added before there was enough of the less poisonous bodies to cause death. This is putting Ehrlich's results in a very crude way but it is an attempt to reduce them to their simplest terms. What takes place is probably very complicated as bodies having very different affinities and toxic powers exist in the same crude toxine. The bodies having the greatest toxic power associated with the greatest affinity for antitoxine Ehrlich would call the toxines proper; the other bodies of less toxic power even if these had greater affinity for antitoxine he would call toxoids. This differentiation between the combining and the toxic capacities of toxines and toxoids is an essential part of the theory. In it is involved the supposed method by which such poisons as those of diphtheria and tetanus act on the animal body. According to this view susceptibility to one of these poisons depends on the capacity of certain cells to combine with the same group in the toxine or toxoid as the antitoxine combines with *in vitro*. This group Ehrlich names the "haptophorous" or combining group. When by this means the toxine is anchored in the cell then the other group which he names the "toxophorous" begins to exert its poisonous action on the other constituents of the cell. That part of the cell which anchors the toxine is compared to the affinities which occur in the "side-chains" of many complex organic substances whose constitution is known. What then

is the antitoxine found in the serum of an animal immunised against such a disease as diphtheria? Weigert¹ has elaborated a view which accounts for the formation of such a substance on the lines of Ehrlich's theory. It has been shown that the cells of the normal brain of an animal susceptible to tetanus manifest a certain capacity of neutralising tetanus toxine. This would be due to the appropriate side-chains present combining with the toxine. These side-chains which are probably specific for each toxine which attacks the brain must have some normal function in the metabolism of the cell. Now the process of active immunisation against such a disease as diphtheria consists in administering to an animal progressively increasing doses of the toxine. The first administration of the toxine saturates certain of these side-chains and thus robs the cell of their ordinary function. The great capacities possessed by the tissue cells generally to make good a damage provided the latter have not exceeded a certain degree comes into play and the cells give rise to new side-chains. As injection follows injection more and more side-chains are saturated, and more and more are produced till what is in reality a hypertrophy of these elements occurs so that now far more are produced than the cell has any need for. They thus become a waste product and are cast off into the lymph and blood and are the antitoxic elements which can be obtained from the latter. Such in brief outline is Ehrlich's theory in so far as it deals with the diphtheria group of poisons.

This group includes not only the poisons of diphtheria and of tetanus but also certain poisons of non-bacterial origin such as ricin, abrin, the snake poisons, scorpion poison, etc. All of these present certain common features and it is convenient to class them together. The following experiments deal chiefly with the tetanus poisons and also to a small degree with three other members of the group. The object of the research was to investigate a few of the properties of certain artificial modifications of the poisons which are probably to be classed as toxoids. Toxoids, as well as toxines, are produced by bacteria in ordinary fluid media but they also are gradually formed from the latter by the action of such agents as heat, light, oxygen, etc. As will be seen they can also be readily and quickly produced artificially by certain chemical agents.

The crude toxines of the tetanus and diphtheria bacilli which were used consisted of filtered bouillon cultures of these organisms—the diphtheria in ordinary bouillon, the tetanus in dextrose bouillon. No

¹ Lubarsch and Ostertag, *Ergebnisse der allgemeinen Pathologie*. Vierter Jahrgang (1897), p. 107.

attempt was made to get the toxine in a more concentrated form, as it is difficult in the course of the necessary manipulations to avoid the formation of much toxoid. During the research the toxic filtrates were kept in the dark in a cool place and were protected from atmospheric influences by being covered by a layer of toluol. Notwithstanding these precautions in the course of months it was found that a marked loss of toxicity took place in some instances. This however did not affect the results as these were of a relative and qualitative nature.

When any quantitative results were aimed at, the M.L.D. of a fresh toxine was determined and the latter used for the experiments. The accounts of the experiments have not been burdened with the details of the dilutions of the toxines often necessary to ensure accuracy in the measurement of the small quantities of fluid involved but such dilutions had constantly to be practised. In fact such a research as the present is very much hampered by the requirements of accuracy on the one hand and by the fact that only a limited volume of fluid can be injected into a guinea-pig on the other. In inoculating the experimental animals this was invariably done hypodermically over the abdomen or sternum. In the experiments with tetanus toxines several different toxic filtrates were employed. These are referred to by the letters A, B, G, E and F.

I. Action of Acids on Tetanus Toxine.

In their classical paper on diphtheria toxine (*Ann. de l'Inst. Pasteur*, III. 273) Roux and Yersin in stating that lactic and tartaric acids cause a loss of its toxicity add, that, if the acid be subsequently neutralised, the toxine recovers a great measure of its activity. This has always appeared to me a very remarkable fact and I resolved to look into the reaction further. An accident to a quantity of diphtheria toxine prepared for this object led me to confine the first observations to a tetanus toxine which it had been intended to use for finding if the reaction were a general one of the group of toxines in which both are as has been said usually placed. This was very fortunate as the definite functional effects occurring in tetanus enable the slightest response to the poison to be recognised,—an advantage not possessed by the diphtheria toxine, in experimenting with which it is often difficult to assign a true value to a slight local inflammation at the point of inoculation.

The first experiment was as follows :

Experiment 1. (a) .25 c.c. of the toxic bouillon A was exposed to the action of .25 c.c. of lactic acid of about half-normal strength for quarter of an hour and the mixture then injected into a guinea-pig of 270 g. Result : Death in 36 hrs.

(b) The same amount of toxine similarly exposed but at the end of the time named the acid was neutralized with sodium carbonate and the mixture injected into a g.p. of 252 g.¹ Result : Death in the same time.

(c) A control animal of 260 g. received .25 c.c. of the toxine and died in the same time.

As dilution of the acid by the fluid of the toxine had to be allowed for, the effective strength of the former was about a quarter normal. In the time allowed for its action it evidently had no appreciable effect on the toxine.

Experiment 2. (a) 1 c.c. of the same toxine as in Exp. 1 was exposed to .5 c.c. of normal lactic acid for $2\frac{3}{4}$ hrs. At the end of that time a quantity of the mixture containing .46 c.c. of the toxine was injected into a g.p. of 468 g. No illness resulted.

(b) At the expiry of the time named some of the same mixture was rendered slightly alkaline with sodium carbonate and an amount containing .47 c.c. of the toxine was injected into a g.p. of 470 g. Result : Death in $3\frac{1}{2}$ days.

(c) A control g.p. of 365 g. received .36 c.c. of the simple toxine and died of acute tetanus in 36 hrs.

Here the effective strength of the acid was one-third normal and the exposure was much longer than in the former experiment. It will be noted that the general result is that which Roux and Yersin state to be the case with diphtheria toxine under similar circumstances, namely, that lactic acid is capable of destroying the toxine but that a certain amount of the poisonous effect returns if the acid be neutralised with alkali. That it is only a certain part of the toxic power that returns is indicated by the fact that while the control animal died in 36 hrs. the animal which died from the effects of the "regenerated" toxine, as it were, did not die for $3\frac{1}{2}$ days. As animals of suitable weight were not available at the time it was considered advisable to regulate the dose to the body-weight in the way that it will be noted was done. Another experiment along the same lines need not be detailed; it entirely confirmed that given.

While the results of these experiments indicated that it was the acid part of the lactic acid molecule that acted on the toxine it was considered advisable, seeing that this body also contains a hydroxyl

¹ The abbreviation "g." stands for gramme or grammes, "g.p." for guinea-pig."

group, to test the action of an acid hydrogen atom in such a simple acid as hydrochloric acid. The latter as compared with lactic acid has of course greater avidity and one would expect its action to be more powerful if the reaction depended on acidity.

Experiment 3. (a) .3 c.c. of the same tetanus toxine was exposed to .15 c.c. normal hydrochloric acid for 20 min. and then injected into a g.p. of 307 g. Result: On the third day there were some spasms but these passed off and the animal completely recovered.

(b) .28 c.c. of the toxine exposed to .14 c.c. of the same acid for the same time; then the acid was neutralised with normal sodium carbonate and injected into a g.p. of 285 g. Result: Death from tetanus on the 6th day.

Experiment 4. The following experiment was performed with a different toxine (M.L.D. for g.p. of 250 g. about .0006 c.c.).

(a) 1 c.c. tetanus toxine B exposed to .5 c.c. normal hydrochloric acid for 25 min. and mixture injected in a g.p. of 263 g. Result: No illness.

(b) 1 c.c. of same toxine similarly exposed and at the end of the time named the acid neutralised with normal sodium carbonate and the whole injected into a g.p. of 275 g. Result: Death in three days.

These experiments show that, like lactic acid, hydrochloric acid also has the power of destroying a certain amount of the toxicity of the toxine but that when the acid is neutralised a return to some extent of the poisonous property results. There can be little doubt also that the action is due to the acid hydrogen atom in the molecule. Further it will be observed that the time required for the more powerful acid to have a definite action was much shorter than in the case of the weaker lactic acid—a fact which supports the idea that it is the acid *qua* acid that is the cause of the reaction.

These experiments establish the fact that it is possible to hold in abeyance the toxicity of tetanus toxine by treating it with an acid. The phenomenon is a very remarkable one and very difficult to understand. It might be thought that if the poisonous properties of a toxine could be made to return by neutralisation of an acid outside the body that the same neutralisation would take place inside the body in consequence of the fact that the blood, lymph, etc. are alkaline. It may be that the very weak alkalinity of the body fluids may make any such action very slow and thus effect that the body has only a very small amount of active toxine to deal with at a time. Unfortunately it was impossible to test the time taken by very weak alkaline fluids to bring out the phenomenon of this "return toxicity," as I may call it, for the amount of fluid would have been such as could not have been injected into a small guinea-pig.

As has been pointed out, according to Ehrlich's theory there are produced by such bacteria as the diphtheria bacillus not only highly poisonous bodies—the toxins, but also bodies of less virulence—the toxoids, and the latter are also developed in the toxins by the action of light, etc. It therefore suggested itself that it might be worth while to enquire whether the bodies which were responsible for the phenomenon of "return toxicity" might not belong to the group of toxoids. As we have seen, one of the features of the latter is that while the toxicity of these bodies may be less than that of the toxine the immunising power possessed by them may be the same. I therefore proceeded to enquire whether the toxins altered by hydrochloric acid possessed any immunising capacity. A preliminary experiment may be given.

Experiment 5. An earlier experiment designed to show the effects of the same amount of acid on different amounts of toxine furnished two guinea-pigs as follows:

(1) This animal had received .5 c.c. of toxine B which had been exposed to .5 c.c. of normal HCl for 20 min. Its weight was 290 g. No illness had resulted.

(2) This animal (Wt. 270 g.) had received 1.5 c.c. of toxine B exposed to .5 c.c. of the same acid for the same time. No illness had resulted.

Eleven days after the first inoculation both received doses of unaltered toxine as follows:

G.P. 1 (Wt. now 352 g.) received .001 Tox. B (M.L.D. .0006 c.c.). Result: Death in 96 hrs.

Control g.p. (Wt. 333 g.) received .001 c.c. Tox. B. Result: Death in 72 hrs.

G.P. 2 (Wt. now 318 g.) received .0025 c.c. Tox. B. Result: Death in 120 hours.

Control g.p. of 378 g. received .003 c.c. Tox. B. Result: Death in 48 hrs.

In this experiment the animal which had received the smaller dose of "HCl toxine" (as the toxine modified by HCl may be called) lived a day longer than the control animal, while that which received a larger amount of the modified toxine survived three days longer than its corresponding control animal. This experiment is quoted because it encouraged the search for more convincing proof. A definite immunisation of three animals by repeated doses of the modified toxine was now attempted.

Experiment 6. Three guinea-pigs were taken weighing 210, 251, 277 g. respectively.

1st injection. 5 c.c. Tox. B was exposed for 20 min. to the action of 2 c.c. of normal HCl, then the mixture was diluted to 20 c.c. and 5 c.c. were injected into each animal. Thus each received about 1.25 c.c. of the toxine which had been subjected to an acid of two-fifths normal strength and at the moment of injection

the acid was of one-tenth normal strength. This latter concentration of acid is rather too much for the subcutaneous tissues of a guinea-pig to stand as in all of these animals slight sloughing occurred at the point of inoculation which, however, soon healed. Subsequent experiments showed that it was necessary to avoid using an acid of greater concentration than one-fifteenth normal, *i.e.* in the case of hydrochloric acid, roughly, a quarter per cent.

2nd injection 18 days later. 1 c.c. Tox. B exposed to .5 c.c. normal HCl for 25 min. then diluted to 15 c.c. and 5 c.c. injected into each animal, *i.e.* allowing for dilution the effective strength of the acid was one-third normal and the strength at the time of injection was one-thirtieth normal: further each animal received one-third of a c.c. of the modified toxine.

3rd injection on the 29th day same as the second.

4th " " 39th " " "

5th " " 49th " " "

After the first injection there never was any local reaction beyond a very slight swelling at the point of inoculation.

On the 61st day after the first injection,—

G.P. 1 (Wt. increased from 210 to 449 g.) received *one* M.L.D. Tox. G.

G.P. 2 (Wt. increased from 251 to 474 g.) received *four* M.L.D. Tox. G.

Neither manifested any symptom of tetanus.

On the 72nd day g.p. 3 (Wt. increased from 277 to 520 g.) received 33 M.L.D. Three days later it had a slight spasm of one leg which continued for about a week and then disappeared.

On the same day a control animal to No. 3 (Wt. 550 g.) received 33 M.L.D. It died of tetanus in less than sixteen hours.

During the whole process of immunisation no symptom of tetanus was manifested in any of the animals. There was thus no doubt that while hydrochloric acid could completely hold in abeyance the poisonous properties of tetanus toxine it did not at the same time entirely remove the immunising action of the latter. The interpretation of this experiment will be discussed later. Meantime, it may be noted regarding it that immunisation here was at least commenced with a toxine containing potentially poisonous action, as the following fact demonstrated.

Experiment 7. About the time when the first injection of Exp. 6 was performed 1 c.c. of the same toxine used in the latter experiment was exposed to .5 c.c. of normal HCl for 20 min., the acid was then neutralised with sodium carbonate and the whole injected into a g.p. of 275 g. Death resulted in 2 days. Here the animal received .25 c.c. less toxine than the guinea-pigs in the immunisation experiment.

In the action of HCl on tetanus toxine there comes a time when the phenomenon of "return toxicity" ceases to be capable of manifestation, as the following experiment shows.

Experiment 8. The general scheme of this experiment was to expose 1 c.c. of toxine G to 1 c.c. of normal HCl for varying times, then to neutralise the acid with normal sodium hydrate and inject the whole into the experimental animals.

Exposure to HCl	No. of Guinea-pig	Weight in grammes	Result
1 $\frac{3}{4}$ hrs.	1	325	Death in 24 hrs.
2 $\frac{1}{2}$ „	2	265	No illness
4 $\frac{1}{2}$ „	3	302	„
6 $\frac{1}{2}$ „	4	310	„

Such being the case it became interesting to enquire whether with complete disappearance of the "return toxicity" the power of immunisation was also lost. The next experiment throws light on this point.

Experiment 9. The scheme was to immunise three guinea-pigs by means of a toxine whose toxicity was so modified by HCl as to be beyond recovery by neutralisation by alkali. That there might be no doubt on this point the poison was exposed for four hours at room temperature to the action of the acid. The weights of the animals at the beginning were 258, 183, 208 g. respectively.

1st injection: 5 c.c. toxine G was exposed to 5 c.c. normal HCl for the time named. The mixture was then rendered very slightly alkaline with normal NaOH and diluted. Of the dilute liquid g.p. 1 received a portion containing two-thirds of a c.c. of the original toxine, g.p. 2 one c.c. toxine, and g.p. 3 one and two-thirds of a c.c. toxine. (This injection was in reality a part of another experiment.)

2nd injection: on the 8th day following, each g.p. received 1 c.c. of the toxine which had been similarly exposed to the acid which had been then similarly neutralised.

This latter procedure was repeated on the 14th, 30th, 35th, and 39th days.

On the 44th day the animals were injected with unaltered toxine G as follows: No. 1 (Wt. now 330 g.) received five minimal lethal doses; No. 2 (Wt. now 317 g.) received one M.L.D.; No. 3 (Wt. now 361 g.) received eight M.L.D. In no case did any symptom of tetanus appear.

A control g.p. of 339 g. which received eight M.L.D. died of acute tetanus in 48 hrs.

This showed that from a virulent toxine a modification could be easily obtained having no toxic properties but which still retained immunising power. The amount of immunity obtained by these methods may, to judge from the tests applied, not have been very great in degree. The last experiment detailed had however shown that immunity could be developed without any inconvenience to the animal employed, for the neutralisation of the acid did away with the risk of causing pathological effects by its strength. This method of immunisation by neutralised "HCl toxine" was therefore taken advantage of for investigating on a larger scale the extent of the protection effected

by modified toxine. The ordinary method of immunisation by gradually increasing doses of the immunising agent was also departed from. The disadvantage of the latter procedure is of course that, in increasing the dose, the investigator has no guide as to what increase in any particular stage it is safe to make. Though this danger was not present when one was working with toxines whose toxicity was entirely destroyed it was considered that many interesting results might be obtained by using all through the immunisation process exactly the same doses of the same toxine.

Experiment 10. Twenty guinea-pigs were taken all of them rather more than half grown so that the body weight might not increase at a very great rate during the immunisation process. The weights in g. were as follows: 543, 555, 580, 575, 505, 452, 533, 425, 545, 555, 450, 447, 505, 510, 710, 450, 517, 713, 465, 546. The toxine used was G, the M.L.D. of which at this time was for a g.p. of 250 grs. about .03 c.c. Each inoculation consisted in the animal receiving hypodermically one-third of a c.c. of the toxine which had been exposed to normal HCl for four hours at the room temperature, the acid at the end of that time being neutralised by sodium hydrate.

All the twenty animals were inoculated on the 1st, 5th, 8th, and 11th days of the experiment. Ten of them received further injections on the 14th, 17th, 21st and 24th days after its commencement. In no case during the immunisation process were any symptoms of tetanus manifested.

There thus resulted two lots of animals one of which had received at nearly equal intervals of time four injections of equal amounts of the same toxine, the second of which had received eight similar injections at similar intervals. The degree of immunity thus obtained could be now enquired into, and especially it could be noted how the two groups differed in the amount of immunity possessed by each. With regard to each group two enquiries were conducted, firstly, What was now for each the fatal dose of tetanus toxine? Secondly, What amount of antitoxic action did the serum of each possess? It was found that the number of animals immunised was too small to enable complete answers to these questions to be given, but nevertheless the results were of an important character.

Experiment 11. Fatal dose of tetanus toxine for Series 1, *i.e.* those treated with four doses of modified toxine.

The toxine used for the test here was a sample known as E, the M.L.D. of which a number of experiments had shown to be .09 c.c. for a g.p. of 250 g. It was thus a weak toxine.

On the 14th day after immunisation had been commenced and the 3rd after the last injection g.p. 1 (555 g.) received 66 M.L.D. No illness resulted.

On the 15th day after immunisation commenced, g.p. 2 (580 g.) received 111 M.L.D. Three days later it was markedly tetanic but from this it recovered.

On the 17th day g.p. 3 (575 g.) received 122 M.L.D. It was markedly tetanic on the following day and died 48 hrs. after inoculation.

The fatal dose for this series thus lay between 111 and 122 simple M.L.D.

Experiment 12. Antitoxic properties of serum of Series 1.

The investigation here was unfortunately hampered by the fact that two of the animals died before any use could be made of them, one from (?) tubercle and another from a diarrhoea which had carried off several animals in the laboratory about this time. Thus only two animals were available for obtaining serum. These were killed by arteriotomy, the blood collected in a cylindrical glass and allowed to stand 24 hrs. in a cool place. The serum was then poured off into a Petri's dish and evaporated to dryness over sulphuric acid *in vacuo*. For use, the desired amount was carefully weighed, dissolved in a measured quantity of .75 % sodium chloride, then .1 c.c. of toxine E was added (*i.e.* just over one fatal dose), the mixture was allowed to stand half-an-hour at the room temperature, and the whole then injected into the test animal.

The two animals whose serum was used in this experiment were killed during the week succeeding the 4th injection of the modified toxine.

No. of Guinea-pig	Weight in grammes	Amount (in grammes) of serum acting on toxine	Result			
1	233	.001	Tetanic	2nd day :	Dead	3rd day
2	235	.010	"	2nd "	"	3rd "
3	240	.050	"	2nd "	"	5th "
4	227	.175	"	4th "	"	7th "
5	275	.275	"	4th "	"	7th "
6	260	.498	"	4th "	Recovered	

The amount of the serum of this series of immune animals necessary to neutralise one lethal dose for a guinea-pig thus lay between 275 and 500 milligrammes. It was unfortunate that a closer approximation could not, for the reasons already given, be arrived at. As it is the condition which ought to be complied with in estimating the strength of a serum, namely, the ascertaining the amount necessary to *completely* neutralise a M.L.D., could not be satisfied. The above experiment however justifies the conclusion that this amount must have been a little over 500 milligrammes. The serum was a very weak one.

Experiment 13. Fatal dose of tetanus toxine for series 2, *i.e.* the animals treated with eight doses of the modified toxine.

The toxine used here was a sample named F and a series of experiments had shown the M.L.D. for a g.p. of 250 g. to be .0008 c.c.

Seven animals could be devoted to this experiment.

No. of Guinea-pig	Weight in grammes	Number of days between commence- ment of immunisation and injection of toxine	No. of M.L.D. injected	Result
1	447	31	100	No illness
2	510	32	200	" "
3	600	35	300	" "
4	629	38	400	" "
5	517	39	600	" "
6	465	39	800	Slight stiffness in one leg for a few days. Ultimate complete recovery
7	713	53	1000	Ditto

Thus the number of animals which could be given up to the elucidation of this point was exhausted before the dose of toxine which would be fatal was reached. It will be noted that in the cases of all the animals except the last the times which elapsed between the commencement of immunisation and the injection of toxine were roughly speaking double those given in the corresponding experiment with Series 1. The results of Exp. 12 as compared with those of Exp. 10 are so remarkable that it may be thought that not sufficient care was bestowed on the determination of the M.L.D. of the two toxines used. It may therefore be said that great care was taken in the matter and that, if anything, the error has been rather in making the figure too large than too small.

Experiment 14. Antitoxic action of the serum in Series 2.

The procedure was that pursued in Exp. 11.

No. of Guinea-pig	Weight in grammes	Amount (in grammes) of serum acting on toxine	Result
1	257	·050	No illness
2	274	·025	" "
3	270	·010	" "
4	260	·005	Slight stiffness for a few days,— complete recovery.
5	259	·001	
6	278	·0005	Ditto
7	265	·00025	Ditto

With this serum therefore a M.L.D. was completely neutralised by 10 milligrammes but not completely by five.

Experiment 15. The object of this experiment was to find out, if possible, in how far the immunising capacities of tetanus toxine were affected by the continued action of hydrochloric acid. The method was to take the same amount of toxine as that used in Exp. 10, but instead of exposing it for four hours to expose it only for half-an-hour. If the acid had been now neutralised the animal would

have run the risk of death from the effect of the return toxicity. The mixture was therefore only diluted to a point at which no evil effects from the acidity would accrue to the animals. Two animals were thus treated and each received four injections of the modified toxine at the same intervals of time as in Exp. 10 and were killed during the week succeeding the fourth injection as in Exp. 12. Amounts of their sera corresponding to the amounts recorded in the latter experiment were tested in the same way with the result that no differences could be observed between the antitoxic properties and those of the sera in Exp. 12. This would indicate that the action of HCl on the immunising capacities of a toxine is very slow as compared with its action on the toxic properties.

Looking now at the results of Experiments 9, 10, 11, 12, 13, we see there are several noteworthy points.

(1) The M.L.D. of toxine G being .03 c.c. and Series 1 being immunised by in all 1.33 c.c., and Series 2 being immunised by 2.66 c.c. of this toxine, it follows that in Series 1 forty-three M.L.D. produced an immunity equal to between 100 and 110 M.L.D., and in Series 2 eighty-six M.L.D. produced an immunity equal to an amount exceeding 1000 M.L.D.

(2) When an animal is immunised by a series of injections of the same amounts of the same toxine and when another animal is immunised by double the number of similar injections the degree of immunity produced in the latter is not double the amount produced in the former in double the time.

To sum up what these experiments show regarding the action of HCl on tetanus toxine we may say that there is a period when the acid weakens but does not destroy the toxicity. This is succeeded by a period when the toxicity is held in abeyance by the acid, but during this time a certain degree of poisonous action can be made to reappear by neutralising the acid by an alkali, and further while the toxicity is in abeyance the power of conferring immunity is present. Finally there is a period when the phenomenon of return toxicity can no longer be elicited, but during the earlier part of which at any rate a very considerable immunising capacity still remains.

The action of hydrochloric acid on other toxins belonging to the same group as the tetanus toxine was investigated though not so thoroughly as in the case of the latter body.

II. *Action of Hydrochloric Acid on Ricin.*

The solution used here was made by taking 20 g. of castor oil seeds, bruising them in a mortar and extracting them for 24 hrs. with 100 c.c. of 10 per cent. sodium chloride. The emulsion was then filtered and a few crystals of thymol were added for purposes of preservation. The M.L.D. of this poisonous fluid was not determined, but one-thirtieth of a c.c. was sufficient to kill a large g.p. in 16 hrs. This toxine was found to be very resistant to the action of the acid as the following experiment will show.

Experiment 16. (a) One-thirtieth c.c. ricin exposed to one-thirtieth c.c. HCl of five times normal strength (i.e. actual effective strength of acid was two and a half times normal) for 6 hrs. at room temperature; the mixture was then injected into a g.p. of 250 g. Result: Death in two days.

(b) Procedure same as (a) but at end of time acid neutralised with normal sodium hydrate and the mixture injected into a g.p. of the same weight. Result: Death in the same time.

(c) Procedure same as (a) but the mixture kept 3 hrs. at 37° C. and the mixture injected into a g.p. of 243 g. Result: Death in 2 days.

(d) Same as (b) but the mixture kept 3 hrs. at 37° C. while the acid was acting. G.P. of 263 g. Result: Death in 3 days.

(e) Same as (a) but mixture kept at 37° C. for 6 hrs. before injection into g.p. of about 280 g. Result: No illness.

(f) Same as (b) but the mixture kept at 37° C. for 6 hrs. while the acid was acting. G.P. of about 280 g. Result: No illness.

In the case of this poison no evidence was forthcoming of the existence of the phenomenon of return toxicity. Only one immunisation experiment has up till now been done.

Experiment 17. A g.p. of 302 g. received one-thirtieth c.c. of the ricin solution which had been exposed to the same amount of five times normal HCl at 37° C. for 4 hrs. No illness resulted and the same process was repeated on the 5th and 10th days thereafter.

On the 72nd day thereafter the animal received 15 c.c. of the ricin solution and never showed any symptoms of illness. A control animal of the same weight died in 16 hours.

This experiment is very interesting, for it will be observed that the result of the immunisation was to make the animal resistant to an amount of the toxine larger than the amount which produced the immunity.

III. *Action of Hydrochloric Acid and of Sodium Hydrate on Abrin.*

An abrin extract was prepared by soaking 20 g. of seeds in 100 c.c. of 10 per cent. NaCl for about a week with repeated bruising and then filtering the extract and adding a little thymol. Here one-twelfth of a c.c. was sufficient to kill a large g.p. in 16 hrs. A preliminary experiment showed that if this amount were exposed to an equal quantity of normal HCl at 37° C. for $3\frac{1}{2}$ hrs. the toxicity was destroyed. The result of the next experiment was at first puzzling.

Experiment 18. (a) One-twelfth c.c. of abrin was exposed to the action of normal HCl for $3\frac{1}{2}$ hrs. at the room temperature; and being diluted was injected into a g.p. of 320 g. Result: Death in 48 hrs.

(b) Procedure the same but the acid neutralised before injection into a g.p. of 311 g. Result: No illness. (It may be explained that in this experiment the acid was not only neutralised but the fluid was rendered slightly alkaline before injection.)

(c) Procedure the same as (a) but exposure $2\frac{1}{2}$ hrs. at room temperature; injection into g.p. of 316 g. Result: Death in 24 hrs.

(d) Procedure the same as in (b) exposure same as in (c); injection into g.p. of 293 g. Result: Ill next day but recovered.

On consideration the only explanation of this result which appeared feasible was that the recovery of the animals in (b) and (d) was due to the slight exposure to alkali destroying the toxicity of the abrin which had been little if at all affected by the previous exposure to the acid. This opened up a new field of inquiry.

Experiment 19. One-twelfth c.c. of abrin extract was exposed to the same amount of normal NaOH at the room temperature for 25 min. It was then injected into a g.p. of 335 g. Result: No illness.

(b) Procedure same as (a) but the alkali just over-neutralised with normal acid; injection into a g.p. of 283 g. Result: No illness.

It may be deduced from these experiments that in the case of abrin the poison is much more susceptible to the action of an alkali than it is to that of a powerful acid.

IV. *Effects of Acids and Alkalies on Diphtheria Toxine.*

The toxine used here was made by the inoculation of a bouillon prepared from meat which had been allowed to become putrid. Its M.L.D. was at first about .01 c.c., but before the experiments were completed it had risen to about .08 c.c.

Experiment 20. (a) .5 c.c. of the toxine was exposed to .5 c.c. of normal HCl for 25 min. and then injected into a g.p. of 518 g. Result: Death in 24 hrs.

(b) Procedure the same but exposure 1 hr. Result: the same.

Evidently if any comparison can be instituted between the action of this acid here and its action as already detailed in the experiments with tetanus toxine the latter is much more susceptible than is the case with diphtheria. The following experiment illustrates this point more forcibly.

Experiment 21. 1 c.c. of the toxine was exposed to the action of 1 c.c. of HCl of twice normal strength (*i.e.* the effective strength was normal), for a period of 4 hrs. A quantity of normal sodium hydrate was added not sufficient to neutralise the acid, in order that the acid originally present might not have an injurious effect on the animal. The whole was then injected into a g.p. of 397 g. Result: Death in 3 days.

(b) The same as (a); g.p. of 315 g. Result: Death in 2 days.

(c) The same as (a) but the acid was just over-neutralised and the fluid was slightly alkaline; g.p. of 384 g. Result: Death in 3 days.

(d) The same as (c); g.p. of 440 g. Result: Death in 3 days.

Thus an acid of considerable concentration is requisite for any effect whatever to be produced on this toxine. The only effect manifest in Experiments 19 and 20 is a delay in the fatal issue in cases where the toxine has been exposed longest to the most concentrated acid. The following experiment showed a way in which the toxicity of the poison may however be very easily destroyed.

Experiment 22. This was an earlier experiment performed before the resistance of the toxine to acid was known. It consisted in exposing the toxine to the action of the acid for different times and then just over-neutralising the acid with alkali.

	Amount of toxine in c.c.	Amount of normal HCl in c.c.	Exposure	Weight of guinea-pig in grammes	Result
(a)	.5	.5	$\frac{1}{2}$ hr.	250	No illness
(b)	.5	.5	1 „	255	Death 4th day
(c)	.5	.5	1½ hrs.	275	„ 24 hrs.
(d)	.5	.5	2 „	263	„ 24 „
(e)	.5	.5	4 „	335	„ 24 „

At first sight these results are perplexing. On consideration however at the time a possible explanation suggested itself. Through a slip, in the case of (a), instead of the acid being just over-neutralised, about .5 c.c. excess of normal sodium hydrate was added and it also happened that as the g.p. was not quite ready for inoculation the mixture stood for a few minutes. The toxine was thus exposed to the action of one-third normal NaOH for a time after having been exposed to the action of the acid. A similar occurrence happened in the case of (b) but in a less degree.

It thus appeared possible that the alkali might have a powerful action on the toxine and this point was accordingly investigated.

Experiment 23. .5 c.c. of the diphtheria toxine was exposed to the action of .5 c.c. of normal sodium hydrate for different times; the mixture was then almost neutralised and after dilution was injected into the experimental animal.

Guinea-pig	Weight in grammes	Exposure	Result
(a)	370	5 min.	Death on the 14th day. (It was doubtful if this was consequent on the inoculation as one or two uninoculated animals in the hutches died at the same time)
(b)	400	15 „	No illness
(c)	405	30 „	„ „

Experiment 24. Procedure the same as in Exp. 22 but strength of alkali was one-tenth normal.

Guinea-pig	Weight in grammes	Exposure	Result
(a)	430	5 min.	Death 48 hrs.
(b)	360	15 „	No illness
(c)	393	30 „	„ „

There is thus no doubt that diphtheria toxine is very susceptible to the action of sodium hydrate. Here as in the case of abrin it is difficult to compare the action of an acid and an alkali but one can say that while the toxine is not very susceptible to the action of the one it is very susceptible to that of the other. As yet no evidence of the existence of the phenomenon of "return toxicity" has been obtained in the case of diphtheria toxine.

It was natural to enquire whether this modified diphtheria toxine had immunising properties. Up to the present only three animals have been treated with the view of settling this point. The experiment was as follows:

Experiment 25. Two guinea-pigs of 395 and 460 g. were taken and each received .3 c.c. of the same toxine as above which had been exposed to the action of .3 c.c. of normal sodium hydrate for 30 min. (the alkali being just under-neutralised with normal HCl before injection). Three days later they received 1 c.c. of the toxine which had been similarly exposed to 1 c.c. of the same NaOH, the subsequent treatment with HCl being the same. They again received 1 c.c. similarly treated on the 5th, 6th, 8th, 11th, 14th, 19th, 24th days after the first injection. On the 26th day one received .5 c.c. of unmodified toxine,—equal at this time to about 25 M.L.D. and suffered no ill effects.

The action of alkali on diphtheria toxine thus while it destroys the poisonous effects does not entirely remove the capacity of immunisation.

Experiment 26. Antitoxic value of the serum of an animal immunised against diphtheria by toxine modified by alkali. On the 26th day the other g.p. of Exp. 24 was killed and its serum tested in the way detailed under Exp. 11 with the following results.

No. of Guinea-pig	Weight in grammes	Amount of serum acting on toxine	Result
1	225	·050	No illness
2	220	·010	" "
3	225	·005	Death in 4 days

The amount of toxine used for mixing with the quantities of the serum detailed was ·08 c.c. This was just over one M.L.D.

The serum thus exhibited considerable antitoxic power.

V. Action of Sodium Hydrate on Tetanus Toxine.

Experiment 27. (a) 1 c.c. of tetanus toxine G was exposed to the action of 1 c.c. of normal sodium carbonate for four hours at room temperature; the alkali was then neutralised with normal HCl and injected into a g.p. of 235 g. Result: Death in two days.

(b) A second animal, weight 273 g., died in the same time.

(c) A third animal (275 g.) similarly treated except that the mixture was kept at a temperature of 37° C. for four hrs. died in 5 days.

(d) A fourth animal (213 g.) treated in the same way as (c) died in 7 days.

There was evidently a slight action by the alkali on the toxine, but as sodium carbonate is a weak body compared with sodium hydrate further experiments were done with the latter agent.

Experiment 28. (a) 1 c.c. toxine F was exposed to the action of 1 c.c. of normal sodium hydrate for 5 min., the alkali was then nearly neutralised with normal acid and the mixture injected into a g.p. of 324 g. Result: No illness.

(b) Same as (a). Exposure 15 min. Weight of g.p. 312 g. Result: No illness.

(c) Same as (a). Exposure 30 min. Weight of g.p. 412 g. Result: No illness.

(d) Same as (a) but the quantity of toxine exposed to the action of deci-normal sodium hydrate for half-an-hour; g.p. of 420 g. Result: Death in 2 days.

(e) Same as (d). Exposure 1 hr. Weight of g.p. 338 g. Result: No illness.

(f) Same as (d). Exposure 2 hrs. Weight of g.p. 370 g. Result: No illness.

It is thus evident that just as a strong acid like hydrochloric acid acts more strongly on the toxine than a weak acid like lactic acid, so a strong alkali like sodium hydrate acts more strongly than the weaker sodium carbonate. An immunisation experiment performed with tetanus toxine modified by NaOH was inconclusive.

The general result of these experiments is to show that acids and alkalis have an important effect in modifying the toxicity of certain toxines. That the modifications thus produced are to be classed with the toxoids of Ehrlich there can be little doubt. They are produced from virulent toxines, they show a toxicity less in degree than the original poisons and, like the toxoids naturally produced, they show a capacity of giving rise to immunity. Ehrlich has shown that there is reason to believe that the natural toxoids have a greater tendency to lose their toxicity than they have to lose their immunising power. There is little doubt that the same persistence of the immunising power exists in the artificial toxoids described. In Experiment 6 the whole immunisation was effected by toxine in which the toxicity was held in abeyance, and in Experiments 9 and 10 the toxicity was completely abolished. Experiment 6, in fact, furnishes corroborative proof of Ehrlich's contention that the toxic and the immunising actions of such a toxine as tetanus depend on different factors in it. Meantime the advantage of using non-poisonous toxoids for the production of immunity may be pointed out. The risks of giving at any particular stage of the process an overdose are thereby avoided. In the immunisation experiments given, the process has not been pressed in any case very far and yet in the case of tetanus very marked results have been obtained. In this connection it may however be pointed out that it is only within the limits of observation that it can be affirmed that for instance the toxine used in Experiment 10 can be said to have completely lost its toxicity. Certainly 5 c.c. was non-toxic but for quantities greater than this no assurance can be given as it is not safe or practicable to inject much more into a small guinea-pig.

Experiment 10 with its resultant experiments 11 to 14 are of importance because the same dose of the modified toxine was used throughout the whole process of immunisation. The very great rise in the immunity during the second period of the immunisation, though the stimuli remained constant, is in favour of Weigert's theory as to antitoxine production being due to a habit into which certain cells get of producing a certain kind of material.

It may be pointed out that the method of immunisation by means of equal doses of toxine given at equal intervals of time offers great advantages. By its means the progress of immunity can be studied and the immunising capacities of various bodies can be compared.

General conclusions.

(1) Tetanus toxine under the influence of hydrochloric acid loses with comparative readiness its virulently poisonous properties. It does not however so readily lose its capacities of producing immunity and when all trace of toxicity has disappeared the capacity of producing immunity still remains. The less poisonous substances produced in the modified toxine are probably of the nature of toxoids.

(2) Tetanus toxine is also susceptible to the action of alkalies such as sodium hydrate and sodium carbonate, under which it again loses its toxicity.

(3) Ricin is very resistant to the action of hydrochloric acid. There is evidence here also that when the toxicity is destroyed the capacity of producing immunity also remains.

(4) Abrin is also resistant to the action of hydrochloric acid but it is relatively susceptible to that of sodium hydrate.

(5) Diphtheria toxine is very resistant to the action of hydrochloric acid but it is relatively susceptible to the action of sodium hydrate. In the case of toxine which through the latter agent has had its toxicity destroyed there still remains evidence of the capacity of producing immunity.

THE UTILITY OF ISOLATION HOSPITALS IN DIMINISHING THE SPREAD OF SCARLET FEVER.

CONSIDERED FROM AN EPIDEMIOLOGICAL STANDPOINT.

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THERE have not been wanting those who have alleged that isolation hospitals have failed to fulfil the object for which they were erected, inasmuch as scarlet fever has during the last few years been prevalent to an exceptional extent. Those who argue thus, are in favour of diminishing expenditure on the erection and maintenance of isolation hospitals, and consider that we must trust to "improved sanitation" for diminishing and possibly in the end annihilating infectious diseases.

The problem deserves consideration, especially as it must be admitted *in limine*, that the enforcement of hospital isolation has not been so successful in diminishing the prevalence of scarlet fever, as might have been anticipated on *à priori* grounds. I have purposely chosen to consider the problem in relation to scarlet fever, as the case that can be made out in favour of hospital isolation for this disease, is very much weaker than for the same measure in diphtheria, enteric fever, and small-pox. The immense good effected both preventively and therapeutically by the hospital treatment of these three diseases is beyond dispute.

What, however, are the facts as regards scarlet fever? These may be gathered from a study of Fig. 1. This plate shows that in former times there were immense oscillations in the death-rate from scarlet fever, and that these have now become so insignificant as to be almost inappreciable on a diagram drawn to the above scale. Thus in 1861 the death-rate from scarlet fever fell to 451, and in 1863 it had risen to 1478 per million of population. In 1866 it had fallen again

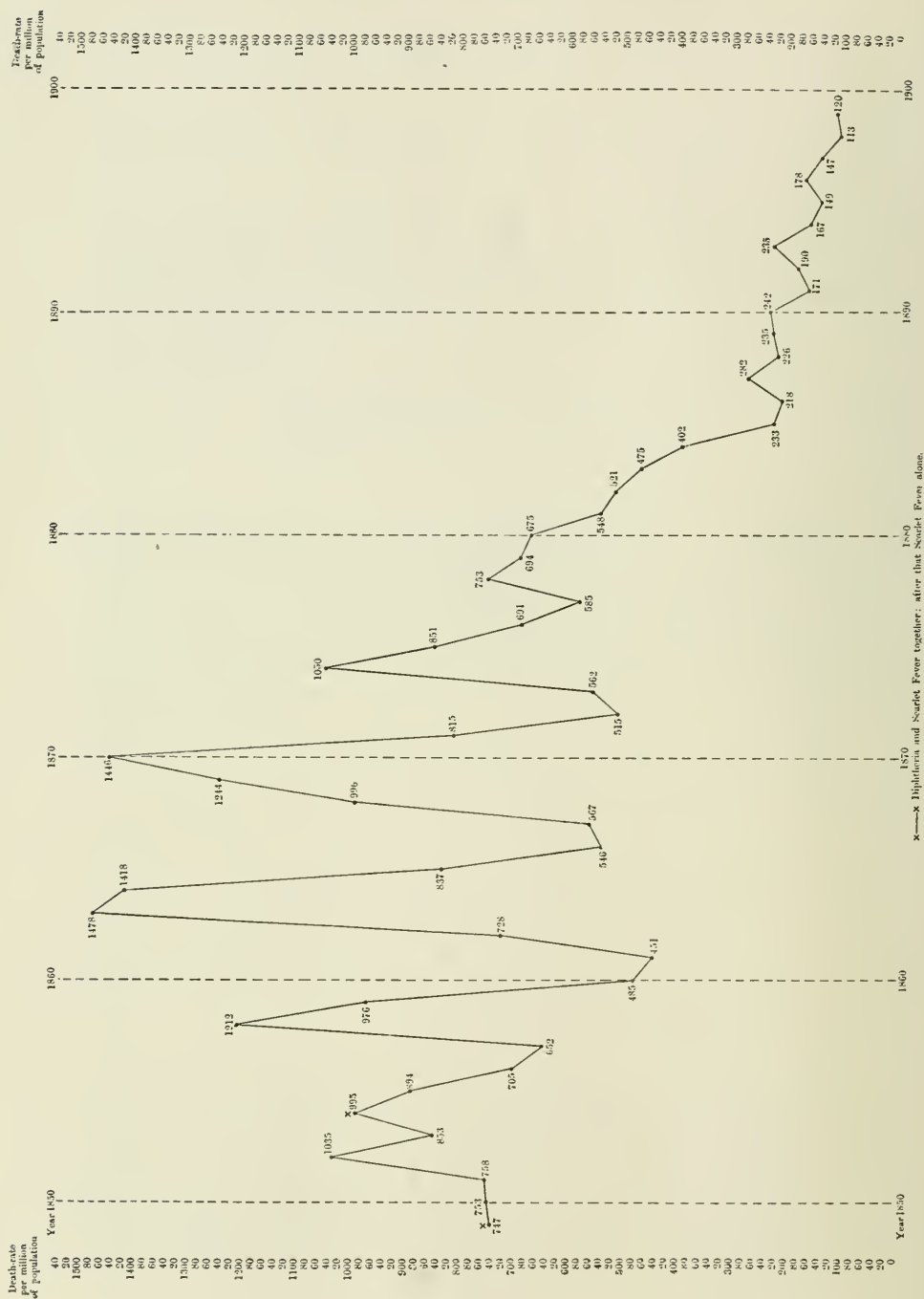


Fig. 1.

to 546 and in 1870 had risen to 1446 per million. Compare this state of matters with the subsequent course of the scarlatinal curve. In 1874—5 a rise to a smaller extent occurred and again in 1878 a still smaller rise. Then a steady fall until 1887, when a trifling rise occurred, the next rise appearing in 1893, when the death-rate only reached 235 per million. It is clear therefore that if death-rates are to be taken as a guide, the scarlatinal returns show a strong case for the continued use of the present preventive measures, among which hospital isolation and disinfection claim an important part. The Infectious Disease (Notification) Act became law in 1889. It cannot be claimed, however, that hospital isolation dates from this year. The Act was, except in London, an adoptive Act, and local authorities have been very slow to adopt it. The present year (1900) is the first in which a similar Act has been made generally compulsory. On the contrary, many districts had local compulsory notification for years before 1889; isolation hospitals throughout the country were becoming more numerous year by year, and the public were being educated as to the necessity for isolation and disinfection, to a rapidly increasing extent from the eighth decade of the 19th century onwards to the present date.

It is alleged, however, that the number of *cases* of scarlet fever has not, even though the total number of deaths has declined. Thus in Bradford the number of cases notified in 1881 (under a local Notification Act) was 2·3, in 1899 it was 9·7 per 1000 of population; in Nottingham under a similar Act 2·3 cases were notified in 1883 and 10·4 per 1000 of population in 1899. (In this latter town 48 per cent. of the total cases were removed to the isolation hospital in 1899, while in 1898 71 per cent. of the total 931 cases were thus removed.) On the strength of figures like the above it has been asserted that notification and isolation of patients in hospitals are useless, and that they may be an impediment to true sanitary progress by diverting attention from sanitary reform.

It cannot be seriously argued that notification *per se* can increase the amount of infection. Nor can the conclusion be seriously resisted that notification, by increasing the sources of information, and making it more complete than it would otherwise be, must aid in the prevention of the notified disease, *assuming that the right measures of prevention are taken*. The question is, are removal of patients to hospital and disinfection of homes after removal the right measures? It has been contended that they are not; and that the increased number of cases of

scarlet fever since notification came into force supports this contention. Such an increase when a sufficiently long series of years is taken, has not however been proved, and is in fact highly improbable. There can be no exact comparison of cases in pre- and post-notification years. Since notification began there has been a steady diminution in the severity of scarlet fever, as evidenced by its case-fatality. Hence it is impracticable to apply present fatality-rates to old statistics of mortality, and argue as to decrease or increase of total number of cases of scarlet fever. Scarlet fever differs from enteric fever and to a less extent from measles in its very great variations of virulence. At one time in the words of Sydenham *hoc morbi nomen, vix enim altius assurgit*; while only a few years later it may again assume intense virulence. The causes of these variations of virulence are imperfectly known. I am not prepared to state that the treatment in recent years of so large a proportion of the total number of cases in isolation hospitals has been the chief determining cause of the change; but that it has helped in producing it, is highly probable. Hence it is a perfectly gratuitous assumption to suppose that since hospital isolation has been largely practised, the number of cases of scarlet fever has increased. Notification has brought them into more prominent public attention, but the statement that they are more numerous than before notification began, is an unproved and unwarrantable assertion. All that we know with certainty is that *the number of deaths from scarlet fever has declined to a most remarkable extent*: of the number of cases we can in the majority of districts only speak since 1890, too short a period to form valid conclusions as to any method of prevention. Even in towns in which notification returns are available for two decades, conclusions as to the efficacy of preventive measures based on the number of cases in successive years must necessarily be fallacious. We cannot secure the essential condition of "*ceteris paribus*."

Light has been thrown on the character of these other conditions by the independent observations of Gresswell and Longstaff¹ who showed that there was an inverse relationship between the amount of scarlet fever and the annual rainfall; Gresswell further suggesting that "not only the rainfall of the year, but also that of prior years, has influence on scarlatina²." Without pursuing this point in detail, it may be briefly stated that all the epidemic peaks shown in Fig. 1 occur in exceptionally dry periods; wet years being always years of little scarlet fever. The

¹ *Trans. Epidem. Soc.* 1880, p. 429.

² *A Contribution to the Natural History of Scarlatina* (Clar. Press, 1890), p. 192.

years 1887, 1893, and 1899 were again years of exceptional drought; and yet how puny are the epidemic peaks of these as compared with those of earlier years! No hygienist has claimed that isolation and disinfection can entirely prevent the occurrence of these epidemic peaks, which are due to cyclical causes beyond our control. All that isolation and disinfection can do in the prevention of scarlet fever is to minimise the possibilities of infection and keep the epidemic peaks down below the height which they would attain in the absence of these measures. A glance at Fig. 1 almost irresistibly suggests that great success has been attained in this direction. The last fifteen years have been almost unexampled for the large proportion of dry years that have occurred, and especially the years from 1892 onwards, as may be seen from the following table.

RAINFALL AT GREENWICH.

Year	Rainfall in inches	Departure from average of 50 years	Accumulated deficiency
1887	19.9	- 4.2	4.2
1888	27.5	+ 3.4	0.8
1889	23.3	- 0.8	1.6
1890	21.9	- 2.2	3.8
1891	25.1	+ 1.0	2.8
1892	22.3	- 1.8	4.6
1893	20.1	- 4.0	8.6
1894	26.9	+ 2.8	5.8
1895	19.7	- 4.4	10.2
1896	22.4	- 1.7	11.9
1897	22.1	- 2.0	13.9
1898	18.9	- 5.3	19.2
1899	22.3	- 1.8	21.0

And yet notwithstanding the fact that the elements have been fighting against them, preventive measures, among which hospital isolation holds an important place, have been associated with the remarkable and almost uninterrupted decline in the death-rate from scarlet fever shown in Fig. 1.

The limitations as well as the extent of the utility of preventive measures against scarlet fever need to be recognised. Even when all practicable preventive measures have been adopted,—and that point is still very distant,—there will, I believe, remain a residuum of infection, the operation of which with our present limited knowledge we cannot prevent, owing to the occurrence of “epidemic influences,” beyond our control. The state of matters may be illustrated diagrammatically.

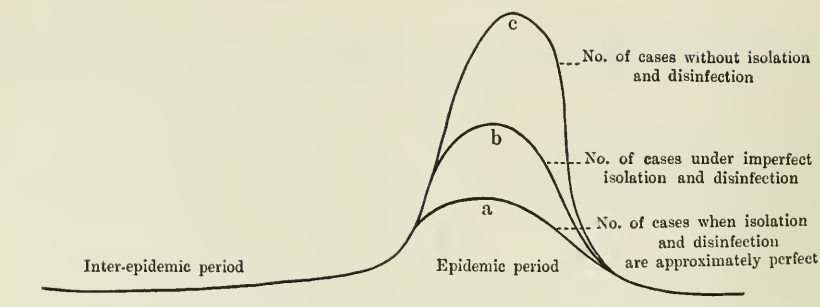


Fig. 2.

I have elsewhere compared the work of the hygienist to that of the staff of a fire-brigade, and the following remarks respecting diphtheria are equally applicable to scarlet fever. "To assume that because we do not yet know how to exterminate diphtheria, or because we cannot hope in our day to be entirely successful in preventing its spread, it is therefore useless to attempt anything, would be as unwise as it would be for a City Council to dismiss their fire-brigade staff and dispose of their fire-preventing apparatus, because the staff had not been successful in at once extinguishing every fire, or because the City Council were impressed with the fact that the present appliances for extinguishing fire are of a very imperfect character¹."

Until better means or supplementary means, the result of fuller knowledge of the natural history of scarlet fever, are devised, it is our obvious duty to persevere with the best known means of preventing the spread of this disease. The determination to persevere on these lines should be strengthened by our present knowledge of the disease. Although the micro-organism causing scarlet fever has not certainly been isolated, we know that the contagium of this disease, like that of other infectious diseases, is particulate; that it can be destroyed by disinfection, and that its dissemination can be prevented by isolation of the sick. It would therefore constitute a sin against knowledge to abstain from preventive action in these directions.

Referring to Fig. 2, the distance between *b* and *c* will obviously increase as preventive measures become more complete, until *a* and *b* coincide in position. We may consider, in conclusion, the reasons which have prevented hitherto, the attainment in any known district, of this ideal condition of things.

¹ *The origin and spread of Pandemic Diphtheria*, 1898, p. 192.

In the first place, hospital and home isolation have never been completely carried out. In very few districts does the percentage of cases removed to isolation hospitals exceed 80: and general experience shows that the isolation of a large proportion of the remaining 20 per cent. is very imperfect.

Secondly, not only have a considerable proportion of notified cases remained un-isolated, but a considerable proportion of the total cases have not been notified; owing to various causes, such as failure to call in medical aid in slight and unrecognised cases, errors of diagnosis, and occasionally neglect to notify. It may be said, then, that notification has been a failure. It is however obviously preferable to have a system of notification in which say 80 per cent. of the total cases of scarlet fever are notified, rather than a total absence of notification, in which information (and the action that can be taken thereon) must necessarily be still more inadequate. Every additional case notified gives an additional opportunity for preventing the spread of the disease by personal infection; and every such notified case can be made a centre of inquiry leading to the detection of unnotified cases, if sanitary administration be active and intelligent.

Thirdly, the best sanitary administration cannot accomplish everything. There must be hearty cooperation on the part of parents and medical practitioners, if efforts to secure early diagnosis and early isolation are to be successful. At this point failure frequently occurs. Cases are notified after being watched for several days, with the natural result that secondary and tertiary cases are common in the same household. The law is much more regardful of the welfare of cattle than of human beings. A doctor need not notify a case of scarlet fever until "he becomes aware" that it is certainly of this nature; a farmer must notify each case *and each suspected case* of foot and mouth disease. A parent need not call in a doctor for every suspected case; and if he does not, he can rarely be proved to have wilfully failed to inform the medical officer of health of an infectious case, as the proof involves the assumption that he or she possesses medical knowledge; and in the absence of such proof the parent cannot be punished for keeping the case secret. This may be said to be an argument against notification: it is rather an argument for improving its machinery. Imperfect information must be better than no information.

The consideration of the relative results obtained by hospital and home treatment of scarlet fever would take us too far afield. My own experience is that hospital-treated have a lower case-fatality than home-

treated cases, notwithstanding the fact that the former include a larger proportion of severe cases than the latter. The occurrence of "return cases" in connection with patients discharged from isolation hospitals is on a relatively small scale. Even though they could not be reduced below the present number, they would not detract to any great extent from the valuable work done by isolation hospitals. That they can be reduced in number by more rigid separation of acute from convalescent, and of uncomplicated from complicated cases is fairly certain.

The preceding remarks may be summarised as follows: scarlet fever being an infectious disease, personal contact between healthy and sick must be prevented, if its spread is to be abated. This can only be done, in connection with the majority of homes, by removal to an isolation hospital. The contagium being particulate and microbic can be destroyed by appropriate disinfection. So far as we know at present isolation and disinfection are the only practicable means for preventing or at least minimising its spread. These measures have not in the past been completely successful, because diagnosis has been defective, carelessness has been prevalent, and isolation has been delayed, and carried out in an insufficient number of cases; and because disinfection has often been effected in a perfunctory manner. Even were all these measures successfully carried out, there would probably remain a residuum of cases, occurring in cyclical waves.

To suppose that the spread of a disease caused by particulate infective material is not diminished by isolation of infective persons and by destruction of infective particles, and to suppose further that the occasional occurrence of "return cases" is more than a small drawback to the good achieved by isolation hospitals, is to strain the facts, and to arrive at a conclusion which is contradicted by our general knowledge of the causation of specific febrile diseases.

A CONTRIBUTION TO THE AETIOLOGY OF PLAGUE.

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THE subject of the following remarks is the mode of spread of bubonic plague in epidemic form as deduced from observations made during the outbreak at Sydney. The nature of the disease, and the whole of the circumstances which accompanied its appearance, having been described at length in my official report, only those points which have a direct bearing on the subject just defined are here mentioned; but a brief preliminary statement of certain local conditions is necessary.

Sydney lies on the eastern coast of Australia in S. Lat. $33^{\circ} 52'$ and E. Long. $151^{\circ} 14'$, and occupies an area of about 256 square miles of sandstone country much broken up by deep fjords. The estimated population of the metropolitan registration district was 438,300 on December 31st, 1899; that of the metropolitan municipalities combined for purposes of sanitary administration was about 456,000. The inhabitants are white; there is among them the sprinkling of coloured people found at every seaport, and a small colony of Chinese which at the last census numbered less than 4000, and which has since diminished. There are no aboriginals, their number being now reduced to less than 8000 in the whole State. The whites are of European extraction; at ages below 15 more than two-thirds are of Australian birth, but at older ages they are largely of immediate European descent or actually of European birth. The members of foreign nations among them are proportionately few. They may be reckoned as English on the whole, and although they inhabit a subtropical climate their institutions and personal habits do not differ much from those of Northern Europe: 293 of the whites were attacked with plague, of whom 95 died; and 10 Chinese, of whom 8 died.

The sea-trade of this port is great, and carried on with almost every part of the world; the total tonnage which entered in 1899 was 2,589,457. Consequently it has been exposed to risk of importing the infection of plague since May, 1894, when the disease first became epidemic at Hong Kong; its distance therefrom by steam is 3 weeks, and 2 lines of steam-vessels furnish a monthly service each; besides which other steam-vessels call regularly during their season, while others still arrive all the year round after touching at various Chinese ports including Hong Kong. Trade with India is almost as great, and with some other infected ports it is regular and considerable; the following are those from which Sydney was chiefly threatened, the date of infection being affixed to each name: Bombay (September, 1896), Calcutta (about or before March, 1898), Mauritius (officially declared February 27th, 1899, but cases had occurred at Port Louis during December, 1898); Kobe, Japan (during December, 1899); Honolulu, H.I. (December, 12th, 1899); and Noumea, New Caledonia (officially declared December 24th, 1899). From 1894 the treatment accorded to all vessels which arrived from plague-infected ports was practically that accorded to clean vessels arriving from cholera-infected ports by the earlier International Conventions; and no vessel ever has arrived which carried or (as far as very careful enquiry and examination of logs revealed) which had carried, either a case or a suspected case of plague. But this practice was varied when the infection of Noumea became known. This port lies but from three-and-a-half to six days' steam away according to the class of vessel making the voyage; and in accordance with the Venice Convention, 1897, ships arriving thence after December 24th were detained at quarantine until expiry of the 12th day from commencing the voyage—2 days having being added to the 10 prescribed by it merely because the French Government had directed its representatives in French colonies to impose 12 days' detention on vessels arriving at their ports. As regards the source whence Sydney immediately derived its infection, all that can be said is that it escaped until shortly after the disease had been admitted to be epidemic at the neighbouring port of Noumea.

We now pass on to enquire whether the first recorded case at Sydney were the first case in fact, as at this present date it is universally believed to have been. Comparison of death-rates under several causes with those for corresponding periods of former years showed that they were rather below the average; and there is no reason at all for suggesting that unobserved plague had caused such fatality as could

impress the register with unusual features. The evidence, therefore, is presumptive. It consists, first, in the ability, public spirit, and number of the medical profession in Sydney; in the racial and social characteristics of the population, and their habit of seeking medical advice on the least occasion; in the prevalence of benefit clubs; and in the number, size, and accessibility of the public hospitals, and of cognate institutions under management either of charitable committees or of the State Government. And, secondly, it consists in the alertness of the medical profession, and the fear felt by the general public. Both had been effectually aroused by news of the infection of Noumea; both had been stimulated on January 15th by an alarm, not clearly seen to be false until long afterwards, of the infection of Adelaide. Lastly, the nature of the first case was publicly announced on January 24th, and was made the subject of lengthy articles in the public press immediately afterwards; and although an interval of 31 days elapsed before the second case became known, only one really doubtful case was referred to the Department of Public Health for diagnosis during this interval; nor at any later time was it professed that cases or doubtful cases were recollected. The exception occurred in a man who presented bilateral, inguinal buboes, in every superficial respect resembling venereal buboes, to the cause of which the most minute enquiry into his habits, occupations, associations, and clinical symptoms, assisted by a prolonged bacteriological investigation, furnished no clue on the one hand, but on the other no support to the suspicion which had made an accurate diagnosis desirable.

Under these circumstances a carman, regularly employed by the Central Wharf Co., was suddenly attacked with severe headache at mid-day on January 19th; 4 hours later he felt some pain in his groin, and found a swelling there. He summoned medical advice on the 20th, and his case was at once reported to the Department. By the 24th rigid proof of the nature of the illness had resulted from the cultural and inoculation tests applied to sanious lymph withdrawn from the swollen gland. This man's business during several months past had been to cart exports from city warehouses to Central Wharf, which was situated rather easterly of the entrance to Darling Harbour; but occasionally to fetch packages which were in course of transhipment from other wharves to that of his employers. It was ascertained from his employers' books, however, that he had had no business at any other wharf for 10 days before the date of attack. Vessels from infected ports had lain at neighbouring wharves during the latter two months

of 1899 and down to January 19th; namely, from Hong Kong, India, Mauritius, and Noumea, and 4 from Hong Kong at Central Wharf itself. He had no business on board any ship, and said that for long he had not boarded any.

The second case was diagnosed from examination of inguinal glands removed after death, and brought to the laboratories, on February 24th, or, as mentioned above, 31 days after identification of the nature of Case I. It was followed by a third the next day, and by others immediately afterwards. The table below shows what was the progress of the epidemic in time, its extent, and the number of deaths:—

TABLE I. *Showing the number of attacks and deaths recorded during each week.*

	Week ending	Cases	Deaths
	20th January	1	0
	27th January	0	0
	3rd February	0	0
	10th February	0	0
	17th February	0	0
1st week	24th February	2	1
2nd "	3rd March	2	1
3rd "	10th March	5	3
4th "	17th March	12	3
5th "	24th March	10	3
6th "	31st March	23	6
7th "	7th April	29	9
8th "	14th April	29	12
9th "	21st April	16	8
10th "	28th April	26	7
11th "	5th May	38	10
12th "	12th May	23	10
13th "	19th May	24	10
14th "	26th May	7	6
15th "	2nd June	17	3
16th "	9th June	4	3
17th "	16th June	10	3
18th "	23rd June	6	0
19th "	30th June	12	3
20th "	7th July	1	0
21st "	14th July	3	0
22nd "	21st July	2	0
23rd "	28th July	0	1
24th "	4th August	0	0
25th "	11th August	1	0
26th "	18th August	0	1

The weekly notifications showed stages of increase, stasis, and decline in the epidemic. During the first 3 weeks only 9 cases occurred, and they were pretty evenly spaced out. During the 4th and 5th weeks 22 were notified. In the 6th the epidemic became established, and so continued for 7 weeks more; two-thirds (208) of the total cases happened during these 8 weeks. The period of decline set in with the 14th, and continued through the 19th week; it was marked by great irregularity in the number of cases notified, the series having been 7, 17, 4, 10, 6 and 12. The epidemic then ceased. The 20th, 21st, and 22nd weeks yielded but 1, 3, and 2 cases, while the last case of all was noted in the course of the 25th week.

It is important to remark that the contagium had its full virulence from the beginning. The mortality was heavy from February 23rd, when Case 2 died; yet among those which immediately followed it were some which did not exceed Case 1 in severity. The only change observed in the contagium was enfeeblement. This began about May 1st; it was recognised on comparing the state of patients on admission to hospital after May 1st, with the state of admission of those received before May 1st, at corresponding dates of illness. Two other points require notice in this connection. One is that whereas it had taken 7 weeks to furnish the first 100 cases, and 5 weeks to furnish the second, 13 weeks elapsed before the 303rd case had been recorded. The other is that no ambulant cases occurred until quite late in the third period. In addition to the general considerations adduced when the actual priority of Case 1 was being discussed and which may be referred to in support of this statement, mention may also be made of the records which show that throughout the epidemic, 221 suspected cases were referred to the Department for diagnosis, many of them by 69 medical men.

The following table, in which the fatality of the disease in the first and second hundreds, and in the remaining 93 (the 10 Chinese being omitted), shows a diminution in accord with the above clinical observation. But it is not cited in proof of it, because on May 13th Yersin-Roux serum became available, and thereafter was steadily used.

TABLE II. *Comparing the fatality of the disease among three arbitrary divisions of the cases which occurred among whites, the ten Chinese having been deducted as shown below.*

						Less Chinese	
						Cases	Deaths
100 cases, Jan. 20, to April 12*,	fatality	37 %	1	1
100 cases, April 12, to May 9*,	fatality	37 %	5	4
93 cases, May 9, to August 9,	fatality	23 %	4	3

* The cases which occurred on these days have been divided.

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The epidemic was not maintained by any of the usual modes of spread. As to direct communication, it appears, after certain cases have been deducted which cannot be justly cited, that it took 276 households to furnish 289 cases; that the number of persons exposed to primary patients was 1752; and that the duration of exposure was as shown in the table:

TABLE III. *Showing the day of illness on which 289 patients and households were removed to isolation. Also the number of secondary cases which occurred after isolation in four households.*

Day of illness	No. of cases	No. of contacts	No. of cases among contacts attacked in isolation
1st day	10	156	—
2nd „	35	170	—
3rd „	77	458	1
4th „	53	320	5
5th „	31	240	—
6th „	27	142	—
7th „	14	66	—
8th „	13	91	—
9th „	7	40	1
10th „	5	24	—
11th „	2	13	—
12th „	1	2	—
13th „	1	1	—
14th „	1	5	—
17th „	1	2	—
20th „	1	1	—
22nd „	1	—	—
46th „	1	—	—
Uncertain date	1	21	—
	282	1752	7

The 7 secondary cases are those of persons who fell ill in isolation. But in fact, 10 households yielded 13 secondary cases; one yielded 4, the others 1 apiece. They occurred under different circumstances: The 7 just mentioned (4 households) began within 3 days of separation from the primary patient and the dwelling; 3 others occurred before removal; while in the remaining 3 the patients fell ill after removal, cleansing of the dwelling, disinfection of its contents, and reoccupation on the 6th day—2 of them while the primary patient still remained in hospital, one after he had rejoined the household. It is clear that the epidemic was not maintained by direct communication and, while the disease can at most have been directly communicated but exceptionally,

the facts concerning these cases raise a strong presumption that it was not so communicated even in them.

As regards mediate communication the foregoing statement also suffices to show that it did not operate within households; but then, it could not have operated outside them either. Inacquaintance of the members of infected households with each other, and separation of the dwellings they occupied by distances which were either literally great or which, in relation to city conditions, were practically great was a marked feature of the earlier cases of the series; and when, as rarely happened, it was found that the patient had been acquainted with one previously attacked, it turned out that the link between them was not family acquaintanceship, but association at the same place of employment. It was impossible to imagine how mediate communication could have operated, at all events on the requisite scale. A great majority of the cases occurred in the families of respectable and provident artisans; this class does not employ laundresses (and as a matter of fact no laundresses were attacked); they do not either choose the first days of an illness which is usually alarming from the beginning to disperse their household goods (and only one pawnbroker's assistant was attacked, quite early in the outbreak). Usually within 10 hours, always in less than 24 hours from notification, the patient and the inhabitants were removed, the infected house was isolated, its contents were in part removed for disinfection, and in other part were cleansed together with the house itself; these latter operations being always completed within 5 days, during which the police interdicted communication with the premises. Unconscious communication of infection to articles of commerce alone remains; here, again, the suddenness of attack and its rapidly incapacitating character must be referred to; in almost all cases it was for this reason hardly possible that infection could have been communicated to them. In short I have no more doubt that the disease was not spread by mediate, than that it was not spread by direct, communication from the sick. The facts negative both beyond reasonable doubt.

It is hardly necessary in these columns to say that there is no ground for supposing that the infection was spread with food and taken by ingestion; the evidence that man can be thus infected with plague, though perhaps good as far as it goes, shows also that he is so infected very rarely. It may be added as regards water, concerning which all that is known is that the bacillus can survive in it for a variable number of days according to its quality—not that animals can be

infected by drinking water carrying *B. pestis*—that Sydney as a whole is supplied from one primary source; that more than one service-reservoir must have been infected to account for occurrence of the disease over the whole area, either from which cases were removed or on which the infection was taken; that the village of Manly where a distinct sub-epidemic occurred has its own separate catchment-area and works; and that the time and place distribution of the infected premises negatives diffusion by this means.

The most important indication of the mode of spread has already been mentioned in connection with inacquaintance between the members of successively infected households. When the total infected houses were charted it appeared that they stood in almost every neighbourhood on the extensive area mentioned at first as being occupied by the city. But as soon as the cases were charted, not in accordance with their place of residence but with their place of employment, it appeared at once that a majority were associated by resort to a particular part of the city; and even in many instances by resort to the same house of business at which they were employed. It was still more singular to note sometimes that they were often employed in quite different departments of the same great establishment, and were hardly acquainted with each other by sight. It became obvious—notwithstanding certain apparent exceptions—that infectivity attached in some way to localities and even to premises; and yet the percentage of attacks among the persons resorting to the locality thus first identified was in all likelihood almost infinitely small. Natural resistance to this infection is probably a negligible condition; it seemed, therefore, that while the infection was in some sense or other localised, resort to the locality involved no great risk. Some special condition rarely existent, seemed to be necessary to permit communication of the virus to man.

However, following this indication the progress of the epidemic in place must be examined as well as may be without maps. What was observed was this. The primary focus of infection having been identified in the manner described, its bounds (never sharply defined, of course) were seen to extend by continuity; it gradually covered to the east the whole of a strip of the city between Darling Harbour and a line of parks a mile long; but, though these spaces remained open to the usual traffic and were traversed by thousands of persons daily, it was there permanently stayed. It never attained the populous city district beyond the parks. On the other hand it did continuously

extend to the south where there was neither water nor open spaces; but again its extension was sharply and permanently limited to the east by a small park, a small reserve on which barracks stand, a cemetery, and thereafter a railway terminus and the permanent way running from it. It spread on a southerly course, thus limited to the east, for two or three miles; and as the distance from the starting point increased the percentage of invaded houses diminished. Now, between the northern end of the last mentioned spaces and the southern end of those first mentioned, is a gap occupied with streets in the usual way. The infective area extended as before by continuity over these streets; and having thus attained an outlet it subsequently spread two or three miles along and in the neighbourhood of the eastern highway. That is one set of clearly distinguishable facts. But there was another. Almost from the beginning outlying cases had occurred which could not then be connected with the original focus; their serial numbers were 4, 6 and 7, the latter representing that household which yielded 5 cases. It will suffice for the present to refer to it alone. Case 7, M. aged 2 years, occurred on March 8th, and was then isolated not merely by position of the house which stood two miles from the original focus at Darling Harbour, but also by its being impossible to connect the patient with the latter in any way. Eleven days later Case 23 happened, not near 7, but about half a mile away to the west. In the neighbourhood of the house occupied by 23 other cases were met with shortly afterwards; and eventually a large area over which the infection spread from east to west instead, as happened on the primary focus, from west to east in the first place, yielded indigenous cases. After spreading westerly it extended northerly. Thus it appeared as though an independent centre had spontaneously arisen, from which, however, the infection was diffused in the same continuous and comparatively slow manner, as from the original centre. A similar event was witnessed in relation to the village of Manly, which has a population of about 3000, and is so placed as to have frontages to the harbour on one side and to the Pacific on the other. It is a favourite resort visited by several thousand people on holidays; its inhabitants are also for the most part engaged in business in Sydney. It is reached by a seven-mile ferry journey which occupies half-an-hour; and can be otherwise reached only by a land road 13 miles long which involves crossing water twice by ferry. On May 1st Case 164, and on May 2nd Case 175 (unconnected with the former) were notified; and thereafter a total of 9 cases occurred on a comparatively small area within a few hundred yards of the main pier, which

itself yielded one case in a person who lived at the refreshment room built on it. Here again the proportion of persons attacked to those habitually crossing the area was exceedingly small; it was also insignificant in relation to the numbers who inhabited the area.

Lastly, while occurrence of single cases in households was above cited as evidence that the disease was not directly or mediately communicated from the sick, it can now be adduced (but with reference only to those houses which yielded what were judged to be indigenous cases) in proof that the infection might and commonly did exist on premises, and yet rarely attacked more than one person. Thus, again, it seems that something more than neighbourhood of man to the source of infection was requisite to diffusion of the disease, and something which (in individual houses) rarely existed. This was even clearer when on business premises which harboured during the daytime from one hundred to several hundred workpeople, only from 2 to 5 persons apiece were attacked, all of whom probably, and a majority certainly, received their infection within them.

The object with which the above observations have been set out in the foregoing manner is to show that as soon as plague occurs among a wholly civilised white population, and therefore under circumstances which permit cognition of all the important facts, it appears at once and clearly that this disease is diffused by none of those means which are effectual in causing (for instance) epidemics of influenza, or of cholera. In its mode of spread it plainly resembled in some important respects the epizootic Tick-fever. No theory could be devised, I think, which would coordinate the observed facts unless it assumed at all events an animated host which should not be human for the infection. In fact two such hosts are requisite; one to diffuse the infection in place, the other to communicate it to man. It is hardly necessary to say what we now believe these two to be; but it is likely that a majority who have not had our practical experience still regard them with the same doubt with which a majority among ourselves regarded them at the beginning of 1900. They are the rat, and a suctorial parasite; and in connection with them the names of Hankin and of Simond must be mentioned, whose papers alone among a large mass of writings appear to me to possess a real and great epidemiological value¹.

¹ EDITORIAL NOTE. The literature on the relation of insects to the dissemination of plague and other diseases has been exhaustively treated by Nuttall (see *Journal of Hygiene*, Vol. I. p. 77 for reference; also *Centralbl. f. Bakteriologie*, xxiii. p. 625, and

Case 1 presented a feature which has not yet been mentioned. The bubo occurred in the lowest gland of the femoral chain on the left side; and in the external retro-malleolar space of the same extremity I observed a circular, purplish-red spot rather less than 3 mm. in diameter. The cuticle, which had been raised, had fallen and was then adherent to the cutis; at one point of the circumference of the spot it was ragged. There were the remains of a very small bleb; and, in accordance with the received opinion that the infection is commonly taken by inoculation, it appeared to indicate the site of inoculation. It also seemed more probable that inoculation had been effected into the delicate skin of this part of the body, and in a situation which was well protected from ordinary injuries of the kind by the boot and sock the patient invariably wore, by a suctorial parasite than in any other way. The inference was drawn that there were already in some part of Sydney rats which had died of plague, and search was at once made for them both by advertisement and in more direct ways. It subsequently appeared that a mortality among the rats at a wharf at which an epizootic first became manifest, had been observed early in January; but nothing was discovered at the time (there being nothing to direct attention to the neighbourhood of this particular wharf) until February 14th. A landing-waiter then reported that he had first observed unusual mortality among the rats there on February 9th or 10th. Sick rats and carcasses were at once collected; and in the course of five days the disease in them had been rigidly proved to be plague.

Dropping, for a moment, the assumption that the epidemic was a consequence of the epizootic, we have to enquire whether it possibly could have been so; that is to say, whether plague-rats became sufficiently diffused over the several areas to account for their infectivity. In the first place, then, the presence of plague was rigidly demonstrated by the usual cultural and inoculation tests, by Dr Frank Tidswell, Micro-biologist to the Department, in 17 rats which were taken on premises at widely separated points of the original focus, where the incidence of the disease was heaviest; in other two taken at the

xxii. p. 87, and *Hygienische Rundschau*, 1899, Vol. ix.). A large series of experiments with *Cimex* and *Pulex* have given uniformly negative results with animals suffering from various septicæmic affections (anthrax, chicken-cholera, mouse-septicaemia, plague). See also Galli-Valerio (*Centralbl. f. Bakteriologie*, 1900, Vol. xxvii. p. 1; 1900, Vol. xxviii. p. 842). The experimental data presented in these publications (1897—1900) do not tend to support Simond's hypothesis.

distant suburbs of Manly and Woollahra; and in one cat sent in from a house in which no case of plague occurred, but which stood on an infected area. Secondly, sick or dead rats in greater or less number were seen by the disinfecting corps at 70 houses in which plague had occurred, and which were scattered over every part of the various infected areas; they were noted (and often seen) by Dr W. G. Armstrong, the medical officer of health for the metropolitan combined sanitary districts. It is worth noting that the residents in these houses often knew nothing about the rats, which were only discovered in course of cleansing operations. Lastly, in many other cases the presence of sick or of dead rats on infected premises was reported by common observers. Briefly, the area over which the epizootic extended coincided with the area over which the epidemic was seen to have extended after all cases had been referred to their probable place of infection—a locality fixed upon after carefully considering the separately recorded facts concerning each of the 303 cases in man.

To gather and record the foregoing observations required merely intelligence, industry, and perseverance, all of which qualities were conspicuously shown by the members of the Staff over which I have the honour and good fortune to preside. It is afterwards that the real difficulty is encountered. How can a septicaemia of the rat be so frequently communicated to man as to give rise to an epidemic? Many must have asked this question and, until an answer was suggested by Simond, must have hesitated to admit more than concurrence between plague in man and in rats. My object being merely to record our experience in this place, I need only express the opinion that the communication is effected by fleas very commonly and, indeed, usually. To be bitten by a suctorial parasite which has lately bitten a plague-rat, is I think, that special circumstance not (in any individual household) commonly encountered, the need for which has been suggested more than once above. It accounts both for the erratic incidence of plague on houses, and for its erratic incidence on the inhabitants of each house.

Our evidence as regards fleas is the following. Excluding Case 1 the patients were searched for the phlyctenulae or the bleb described as occasionally resulting by Simond; but this search was not systematic. Phlyctenulae were noted on the area of skin drained by the gland which furnished the primary bubo in 6 cases; this was but a small proportion, however, of those who were examined. Two of these phlyctenulae were still surmounted with a minute unbroken vesicle. From each smears

were made; and in one a bacillus morphologically resembling *B. pestis* was seen in small numbers. Secondly, narcotised fleas taken from plague-rats were examined in small number by the Micro-biologist, and in one *B. pestis* was found; in this case its identity was proved by inoculation into a guinea-pig. Nine fleas removed from rats were referred to the Government Entomologist for identification; he pronounced two of them to be *P. serratriceps*, the remainder *P. fasciatus*—described by Bosc as the rat-flea, as far back as 1801.

We are now at liberty to revert to Case 7, which has been left unexplained so far. The cottage in which it occurred stood within a couple of hundred yards of a place where the refuse of the city is still dumped, and among it much rotten fruit and other vegetable matter from the infested wharf and others adjoining it. It is on every ground probable that the carcasses of deceased plague-rats were thus carried to the dump, and there devoured by the horde infesting it. At all events dead rats were found after attack of Case 7 in a little outhouse attached to the cottage where the children of the family habitually played; and it happened that the only persons attacked were 3 other young children who alone frequented the outhouse, and their father who cleaned it. The premises moreover were found by the disinfecting corps to be infested with fleas in quite extraordinary number; and the bodies of the younger children were almost literally covered with their punctures. Case 4, it may now also be pointed out, worked for a hay and straw dealer; the patient himself had not for a fortnight at least been near Darling Harbour. But his employer got his supplies from a wharf at back of the house occupied by Case 2 (and this patient had removed 5 dead rats from a water-closet 2 or 3 days before his attack), where the bales of hay, etc., often lay for some days before being removed. Rats were probably conveyed to Manly by ferry-boats, which daily carry both provisions and produce from the same set of wharves; one of the cases happened at the pier, where many dead rats were found, and all the remainder either in persons who frequented the pier, or who lived in houses within two or three hundred yards of it.

The epizootic was first manifested at one of a line of wharves frequented by ships coming from foreign ports, and among them were some from ports known to be infected, including the port of Noumea (New Caledonia). It seems most probable by far that it began in the landing of plague-rats. The alternative seems to be importation of the infection with merchandise, communication of it to the locality, and passage thence to rats. But, while many cities have for years past

daily received immense quantities of merchandise shipped at, and even coming from, epidemic areas, and yet have for the most part escaped, it never has been satisfactorily shown that plague resulted from importation of trade under circumstances which excluded the intervention of rats, or of insects, or of both. Besides, in the present case it has to be supposed that infection which neither attacked susceptible rats on board (for as there was nothing to prevent the ship-rats from landing, if the contrary be supposed *cadit questio*), nor equally susceptible men ashore, did nevertheless infect shore-rats after a sojourn in the soil. Introduction of a hypothetical soil-stage seems superfluous, although no doubt the bacillus can rest in soil.

The notion that plague can be epidemically diffused by agency of the soil has received unacknowledged support, in all probability, from the preponderating occurrence of groin-buboes among the bare-footed population of the East. But the facts do not at all support it. Of those of our 303 patients who exhibited buboes at all, namely 286, no less than 73 per cent. had them in the region of the groin, and nowhere else. Yet the inhabitants of Sydney no more go barefoot than do the inhabitants of London.

I conclude these remarks with a word or two on management of a current epidemic. The word "contact" is much in use; it has a certain convenience, but unfortunately no defined meaning. It does not necessarily mean one who has lately been associated with a plague patient. It means one who has been exposed in more or less close association with plague-rats within five days past (but sometimes with a septicaemic case in man, or with a person dying or dead of plague in any form). If the case probably arose within the house at which it has been discovered, it will be prudent, and it may be necessary, to remove the contacts until the premises have been cleansed. Evacuation of infected localities is the only measure which can be proved to have been useful in India or elsewhere, and I have no doubt of the reason. In a civilized community isolation of contacts must not however be indiscriminately enforced. The patient has often taken the infection away from home, and in fact his illness predicates nothing of the rest of the household.

The question whether the sick must be isolated is to be discussed on different grounds. In the first place, plague can be communicated from the sick, though epidemics are not maintained by that means. Primary plague pneumonia is infectious; secondary pneumonia is not uncommon, and the sputa then often carry the bacillus; discharges

from buboes always carry the bacillus at first, and according to Calmette and Salimbeni generally continue to yield it in viable (though perhaps not necessarily in virulent) form for many days; the solid and liquid excreta carry it, at all events when submucous haemorrhages discharge into bladder or bowels. Infectious discharges should not be turned into sewers except after disinfection; but special care to disinfect them should be taken with plague, because of the possibility that rats may contract the disease from them in the sewers. I believe this possibility has not yet been experimentally investigated; but Indian records are not wanting in instances which, if they do not prove that plague in man has preceded infection of the local rats, yet suggest that this may sometimes have been the true sequence of events. So that both for the sake of preventing those cases which would be likely to arise occasionally by direct communication—a misfortune which would affect only the household to which it happened, as well as for avoiding the risk of infecting sewer-rats with the disease—which would be a public misfortune, there is ample reason for collecting and destroying everything thrown off by the diseased body. That Authority would be imprudent which left this to the care of individual householders. All the sick must be removed to isolation whenever possible; when it is not possible they should be placed in charge of nurses responsible to the authority and (in all matters relating to prevention) under direction of its own Medical Staff.

ON THE INFLUENCE OF BORIC ACID AND BORAX UPON THE GENERAL METABOLISM OF CHILDREN.

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BOTH boric acid and borax are extensively used as food preservatives, and much interest attaches to the question of their influence if any upon the general nutrition of the consumer, especially children. Although this method of preserving food has existed for a comparatively long period there seems no agreement as to the harmfulness or otherwise of these substances when taken with a mixed diet.

The method most valuable for affording us reliable data upon which to base conclusions in this connection is that of comparative metabolic observations on the human subject, extending over a considerable period, and this method we have adopted.

Before entering fully into our own work in this direction we shall briefly criticise the data from which up to the present the conclusions concerning the harmfulness or otherwise of boric acid and borax as food preservatives have been based.

Literature. The literature may be summarised as consisting of, (I.) Experiments made on the influence of these substances upon digestions *in vitro*, (II.) Experiments made on animals as to the effect of prolonged small doses upon their general health and metabolism, (III.) General action on man and one metabolic experiment made on one man.

I. *Experiments made upon the influence of boric acid and borax upon digestions in vitro.*

Comparative qualitative experiments have been carried out in this connection by Hehner¹, Weber², F. J. Allen³, Cripps⁴, Leffmann⁵, Liebreich⁶, Halliburton⁷. Quantitative experiments have also been made by Chittenden⁸, Maybery and Goldsmith⁹, Rideal and Foulerton¹⁰, and Liebreich¹¹.

The outcome of the quantitative experiments which confirm the qualitative ones may shortly be summarised as follows:

Salivary Digestion: Boric acid favours the amylolytic action of saliva (Chittenden). Borax on the other hand has an inhibitory action on the conversion of starch by saliva (Weber, Chittenden, Rideal and Foulerton, Liebreich). This latter effect is shown by Liebreich to be an alkali-action.

Rennet Action: Boric acid either has no influence upon the action of rennet upon milk (Cripps, Halliburton) or hastens it (F. J. Allen). Borax, according to the concentration, delays or prevents rennet action (F. J. Allen, Halliburton). By the addition of small quantities of calcium chloride, however, the rennet action takes place in the presence of borax (Allen). It is interesting in this connection to note that sodium chloride has the same action as borax¹².

Gastric Digestion: Boric acid in large doses favours gastric proteolysis¹³ (Chittenden). Borax in small doses has also a slight accelerating action (Chittenden, Rideal and Foulerton), whilst in large doses according to the increasing alkalinity it has a retarding effect (Chittenden).

Pancreatic Digestion: (a) Proteolysis. Borax in small and large doses, proportionally to its concentration, stimulates markedly pancreatic proteolysis (Chittenden). Boric acid (and boric mixture) have

¹ *Analyst*, 1891, p. 126.

² *Journ. Americ. Chem. Soc.*, 1892, p. 4.

³ *Lancet*, 1896 (i.), p. 1516.

⁴ *Analyst*, 1897, xxii., p. 182.

⁵ *Journ. Franklin Inst.*, 1899, p. 103.

⁶ *Vierteljahrsschr. f. gerichtl. Medicin*, 1900, p. 83.

⁷ *Brit. Med. Journ.*, 1900, ii, p. 1.

⁸ *Dietetic and Hygienic Gazette*, 1893, p. 25.

⁹ *Journ. Americ. Chem. Soc.*, 1897, p. 889.

¹⁰ *Public Health*, 1899, No. 3, p. 554.

¹¹ *loc. cit.*

¹² Ringer, *Journ. of Physiol.* 1895, p. 425.

¹³ Maybery and Goldsmith's results, apparently in conflict with the above statement, are however vitiated by the variation in their control experiments.

a distinct inhibitory action. (b) Amylolysis. Borax mixture exerts a retarding action on the conversion of starch by commercial pancreatic extract (Rideal and Foulerton). Borax itself has a slight retarding action, whilst boric acid has no action (Liebreich).

The results of the above observers seem to justify the conclusion that (the radicle of) boric acid and borax as such exerts no specific action, the effect in each case being referable to the acid, or alkali moiety, all digestions taking place in an acid medium being inhibited by borax, those occurring in an alkaline medium by boric acid.

II. *Experiments made upon animals.*

(a) Effect of prolonged small doses. Animals have been fed for different periods with food containing various quantities of borax and boric acid by several observers (Neumann¹, Annett², Rideal³, Liebreich⁴). The outcome of these experiments is shortly that boric acid and borax given in small doses for prolonged periods have no influence on the general health of animals (Neumann, Rideal, Liebreich).

Excessive doses (10 grammes or more according to body weight) produced transient nausea and vomiting.

To elucidate the question of the effect of boric acid upon young animals⁵ a series of experiments were made upon young sucking-pigs by A. D. Hall and H. S. Hammond in collaboration with ourselves at the South-Eastern Agricultural College, Wye. These observations⁶ show that 0·2 to 2·4 grammes boric acid per diem continued for seven weeks added to a mixed weighed diet had no influence upon the live weight, growth, and general health of the animals.

(b) Metabolic experiments on animals have been made by Cyon⁷, Gruber⁸, Chittenden and Gies⁹, and Liebreich¹⁰.

Chittenden and Gies' experiments are very complete and accurate, and in their paper will be found a detailed criticism of the earlier less complete work. The chief conclusions from Chittenden and Gies' work are best given in their own words shortly as follows:

¹ *Arch. f. exp. Path. u. Pharm.* 1881, p. 149.

² *Lancet*, 1899, II. p. 1282.

³ *Lancet*, 1900, I. p. 228.

⁴ *loc. cit.*

⁵ The experiments of Annett (*loc. cit.*) in this regard cannot be considered conclusive. *Vide* Liebreich (*Lancet*, 1900, I. p. 13), and Rideal (*loc. cit.*).

⁶ These observations will be published *in extenso* elsewhere.

⁷ *Comptes rendus*, 1878, T. LXXXVII. p. 845.

⁸ *Zeitschr. f. Biologie*, 1880, p. 198.

⁹ *American Journ. of Physiol.* 1898, p. 1.

¹⁰ *loc. cit.*

Moderate doses of borax up to 5 grammes per day, when continued for some time, are without influence upon proteid metabolism and do not exert any influence upon the general nutritional changes of the body. Large doses of borax, 5—10 grammes daily, have a direct stimulating effect upon proteid metabolism. They tend to retard somewhat the assimilation of proteid and fatty foods, increasing notably the weight of the faeces and their contents of nitrogen and fat.

Boric acid in doses up to 3 grammes per day is practically without influence upon proteid metabolism and general nutrition.

Neither boric acid nor borax affects intestinal putrefaction.

Liebreich's experiment with borax on a dog confirmed Chittenden and Gies' results.

III. *General action on man, etc.*

There is abundant evidence that boric acid and borax can be taken by man in considerable doses over long periods in the food, or by itself without producing any toxic effect¹. It is also, however, definitely established that in certain patients medicinal doses (1 gramme two or three times a day) give rise to transient erythematous eruptions after relatively short periods. It is, however, to be noted that these eruptions were, so far as we are aware, invariably produced by the use of boric acid or borax as a drug, no case being reported of the occurrence of rashes caused by these substances in food. How far these rashes depend on idiosyncrasy and are comparable to those produced by shellfish, strawberries, etc., is outside our purpose to discuss here.

The only metabolic experiment made on man published up till now is one by Forster². The action of boric acid alone on one man was examined. Two relatively short series of experiments were made. In the first 3 grammes of boric acid were given daily for three days with a mixed diet. The second experiment consisted of two periods of two days each in which the subject, an adult man, took 1·5 and 0·5 grammes of boric acid per diem respectively with a milk diet. Forster's conclusions, which appear to have been much more cautiously expressed by himself than by his abstractors, are that boric acid has no influence on proteid metabolism and fat assimilation. He found,

¹ R. Virchow, *Berl. klin. Wochenschr.* No. 1, 1884; Gaucher, *Bull. Méd.*, 1890, No. 46 (quoted from Lehmann, *Die Methoden der prakt. Hygiene*, Wiesbaden, 1901, p. 305), amongst many others.

² *Arch. f. Hygiene*, 1884, II. p. 75.

however, that the total quantity of faeces and their nitrogen and phosphorus percentage were slightly increased. Forster is inclined to ascribe this to a slightly diminished assimilation, together with an increased epithelial and mucous excretion from the intestine. (This latter, however, he regards as being purely hypothetical.) He also concludes that boric acid may possibly exert an intestinal antiseptic action, as indicated by the slight decrease of the ethereal sulphates in the urine.

Lehmann in his *Methoden der prakt. Hygiene*, 1901, states that the results of an observation made under his direction by K. Mann upon the latter's own metabolism (not yet published) did not confirm those of Forster.

GENERAL ARRANGEMENT AND METHOD OF OUR OBSERVATIONS.

Before entering into the details especially relevant to our own observations it might be well to recapitulate briefly the general principles of metabolic experiments. They consist in the exact estimation of the quantity of food and its various constituents during a given period, and the estimation during the same period of the total excreta and their constituents, chiefly with regard to Nitrogen, Phosphorus, Fat, etc. By this means we get valuable information with regard to the assimilation of these substances by and their retention in the body. We should like to point out however that there is a slight fallacy in this reasoning, in that our knowledge of the origin of faecal nitrogen is somewhat limited. According to Prausnitz¹ the whole of the nitrogen in the faeces arises not from the unabsorbed nitrogen of the food, but from the intestinal secretion (epithelial cells, etc.). The value of the metabolic method is not to any extent affected by this, in so far as all nitrogen excreted by the faeces must be regarded as lost to the body, and its subtraction from the quantity of nitrogen ingested gives us the quantity of nitrogen retained. It must, however, be observed at once as has been emphasised by Pawlow² that the results of these experiments give us no absolute information with regard to the actual digestibility of any given food, in so far as we are left by them in complete ignorance of the amount of energy spent by the organism in producing the observed effect. Provided the organism is equal to the occasion an indigestible food might be as

¹ *Zeitschr. f. Biologie*, 1897, p. 287.

² *Die Arbeit der Verdauungsdrüsen*, Wiesbaden, 1898.

well assimilated and retained as a digestible one, but to produce this result an additional output of energy would be required. If this additional output of energy were relatively small we should probably have no indications with regard to it, but were it relatively large, or in other words were the difference in the digestibility of the foods in question great, we should probably find that the body weight or the general health of the person under observation would be affected, and this effect would be the more noticeable the longer the period of observation, and the more sensitive the person chosen. From these reasons we thought it advisable to allow our observations on general principles to extend over comparatively long periods, and to take what were *a priori* to be regarded as relatively sensitive reagents, viz. children both robust and delicate, and to observe minutely during the various periods their general health and behaviour. From another standpoint children had, in this connection, an additional interest, on account of the fact that milk forms so large a proportion of their diet, and it is to milk that boric acid and borax as preservatives are generally added.

Our observations were made upon three children, two of whom (boys) might be regarded as typically healthy, and were aged $2\frac{1}{2}$ and 5 years, the third child (girl aged 4 years) was delicate, being convalescent from pneumonia. We shall refer to the children subsequently as *A*, *B*, and *C*, respectively. During the whole period the children were under our perpetual observation, and absolute control was kept over all ingesta, which were accurately weighed by us, and excreta, which were collected in diurnal periods without loss. The general conditions of their life remained constant, they were kept for some time before the "fore period" of the observation began under identical conditions to those obtaining during the observation, they took each day the same amount of exercise, and their habits were in every respect regular. The research was carried out during the months of May and June, and extended in the case of *B* and *C* over a period of 22 days, in the case of *A* over one of 25 days. Each period was subdivided into four, a fore, a boric acid, a borax, and an after period. The relative lengths of these periods will be seen from the tables. The children had a mixed diet. With regard to the quantities of the different food stuffs we were guided at first by the work of Camerer¹. This was subsequently modified to a small extent by our own observations concerning the establishment of nitrogenous equilibrium in which

¹ *Der Stoffwechsel des Kindes*. Tübingen, 1896.

the children were approximately placed before the fore period began. Every article of food was carefully analysed, with regard to its percentage composition, and in no case were so called average figures taken.

In order to minimise the amount of analytical work entailed by this method the three children were supplied from the same stock of foods, which were taken originally in as large a quantity as was consistent with their keeping properties. To this end pasteurized milk was supplied to us in bottles, each lot of bottles being taken from the same churn¹. Each lot of meat lasted for about four days, lean beef was usually taken, and the whole stock minced, a sample of this was then analysed.

The following table shows the percentage composition of the foods used:—

TABLE I.
SHOWING THE PERCENTAGE COMPOSITION OF THE FOODS.

—	Specific gravity	Water %	Fat %	Total carbohydrates %	Nitrogen %	Phosphoric acid %	Ash %
Milk I ...	1·0310	88·65	3·00	Lactose 4·61	0·52	0·23	0·69
„ II ...	1·0310	86·63	4·20	4·60	0·53	0·27	0·69
„ III ...	1·0330	87·61	3·10	4·94	0·56	0·27	0·72
Bread I ...	—	37·90	0·14	Dextrose 55·97	1·20	0·16	0·79
„ II ...	—	37·90	0·13	55·97	1·20	0·16	0·79
„ III ...	—	36·90	0·18	58·02	1·13	0·15	0·48
Butter I ...	—	12·68	86·00	Lactose 0·14	0·11	—	0·50
„ II ...	—	14·56	84·37	0·29	0·08	—	0·29
„ III ...	—	12·43	85·69	0·16	0·19	—	0·51
Meat I ...	—	72·85	2·58	Dextrose —	3·88	0·45	1·16
„ II ...	—	69·22	10·23	—	3·12	0·42	1·08
„ III ...	—	73·70	2·93	—	3·33	0·43	1·17
„ IV ...	—	73·91	2·74	—	3·34	0·39	1·14
Apple Compote I	—	63·02	—	Dextrose 29·84	0·06	0·04	0·33
„ „ II	—	75·80	—	21·80	0·05	0·04	0·34
„ „ III	—	63·18	—	31·08	0·13	0·06	0·43
Toffee ...	—	3·14	4·33	Dextrose 76·95	0·03	—	—

¹ For this we are indebted to Mr Droop Richmond of the Aylesbury Dairy Co.

The excreta were collected without loss in twenty-four hour periods, from 8 a.m. to 8 a.m., and worked up the same day. The faeces were weighed in their normal state each day, small quantities of acid added when necessary, and subsequently evaporated on a water-bath. When dry they were finely powdered and analysed. The faeces belonging to each period were separated by means of the administration of powdered charcoal.

Methods of Analysis. All nitrogen estimations were made by Gunning's¹ modification of Kjeldahl's method. It was found advantageous, especially in the analysis of the faeces, to add a few crystals of copper sulphate to the mixture of sulphuric acid and potassium sulphate, as by this means a very rapid and quiet oxidation was obtained. Two methods of *phosphorus* estimation were used. In food, faeces, and urine, the total phosphorus was estimated by Neumann-Keller's method², viz. by oxidation in a Kjeldahl's flask by means of nitric acid and ammonium nitrate, subsequent precipitation with molybdic solution etc., and weighing as magnesium pyrophosphate. For the estimation of lecithin phosphorus in the ethereal extract of the faeces the usual process was used, oxidation by means of a mixture of sodium carbonate and nitrate and subsequent estimation of the phosphorus as before. The carbohydrates were estimated gravimetrically as dextrose or lactose by means of Fehling's solution. The *fats*, which term includes all the ether soluble substances, were estimated by extraction in Soxhlet's apparatus, after previous treatment with alcohol according to E. V. Voit³. *Lecithin* was estimated by multiplying the phosphorus figure obtained from the filtered ethereal extract of the faeces with the factor 7.27, corresponding to distearyl-lecithin. The *uric acid* was determined by our own modification of Hopkins' method⁴. The total and ethereal sulphuric acids were estimated according to Baumann's method⁵.

¹ *Zeitschr. für analyt. Chemie*, 1889, p. 89.

² *Zeitschr. für physiol. Chemie*, xxix. p. 151.

³ *Zeitschr. für Biol.*, xxvii. p. 555.

⁴ *Centralbl. f. Physiol.*, 1897, p. 434. It has been shown by the most recent workers that the initial precipitation of uric acid by means of ammonium chloride is just as reliable as the more complicated method of Salkowski-Ludwig when certain conditions are observed. Ritter, Folin, Wörner, etc.

⁵ *Zeitschr. f. physiol. Chem.*, i. 70. See also Neubauer and Vogel, *Analyse des Harns*, p. 724.

TABLE II.

SHOWING THE INFLUENCE OF BORIC ACID AND BORAX

PERIOD	—	Date	Dose g	URINE						
				Quantity c.c.	Reaction	Specific gravity	Total sulphuric acid g	Ethereal sulphuric acid g	Uric acid g	Nitrogen g
FORE PERIOD		9 V		240	Amphoteric	1·0290	0·6845	0·0322	0·1350	4·52
		10 "		310	Acid	1·0268	0·8841	0·0415	0·1744	5·42
		11 "		345	Amphoteric	1·0230	0·9839	0·0462	0·1941	5·15
		12 "		445	"	1·0195	1·2691	0·0596	0·2503	5·59
		13 "		440	Acid	1·0180	1·2551	0·0590	0·2475	5·13
		14 "		325	"	1·0235	0·9373	0·0442	0·0683	4·95
		15 "		285	Amphoteric	1·0226	0·8220	0·0388	0·0599	4·21
		16 "		245	"	1·0238	0·7066	0·0333	0·0515	4·11
	Total	8 days		2,635			7·5426	0·3548	1·1810	39·08
	Average	1 day		325		1·0233	0·9428	0·0444	0·1476	4·88
BORIC ACID PERIOD		17 V	0·50	360	Amphoteric	1·0210	1·0123	0·0561	0·1728	4·91
		18 "	0·50	315	"	1·0258	0·9064	0·0491	0·1512	5·16
		19 "	0·50	300	"	1·0228	0·8436	0·0468	0·1440	4·85
		20 "	0·66	425	"	1·0205	1·1957	0·0663	0·2040	5·69
		21 "	0·66	360	"	1·0196	1·0123	0·0561	0·1728	4·01
		22 "	0·66	410	"	1·0205	1·0953	0·0558	0·2091	6·49
		23 "	1·00	430	"	1·0230	1·1489	0·0584	0·2193	6·75
	Total	7 days	4·48	2,600			7·2145	0·3886	1·2732	37·86
	Average	1 day	0·64	370		1·0218	1·0306	0·0555	0·1819	5·41
BORAX PERIOD		24 V	1·5	350	Acid	1·0228	0·9240	0·0574	0·1733	5·17
		25 "	1·5	320	"	1·0235	0·8448	0·0529	1·1585	5·00
		26 "	1·5	300	"	1·0225	0·7920	0·0492	0·1485	4·52
		27 "	1·5	450	"	1·0192	1·1188	0·0738	0·2228	4·12
		28 "	1·5	270	"	1·0263	0·7128	0·0443	0·1337	4·31
	Total	5 days	7·5	1,690			4·3924	0·2776	0·8367	23·12
	Average	1 day	1·5	338		1·0228	0·8785	0·0555	0·1673	4·62
AFTER PERIOD		29 V		355	Acid	1·0219	0·8975	0·0497	0·1438	5·01
		30 "		335	"	1·0228	0·8469	0·0469	0·1357	4·95
		31 "		570	"	1·0188	1·4410	0·0798	0·2308	7·10
		1 VI		435	"	1·0165	1·0996	0·0609	0·1762	4·86
		2 "		450	"	1·0205	1·1376	0·0630	0·1823	5·55
	Total	5 days		2,145			5·4226	0·3003	0·8698	27·47
	Average	1 day		429		1·0201	1·0845	0·0600	0·1737	5·49

TABLE II.

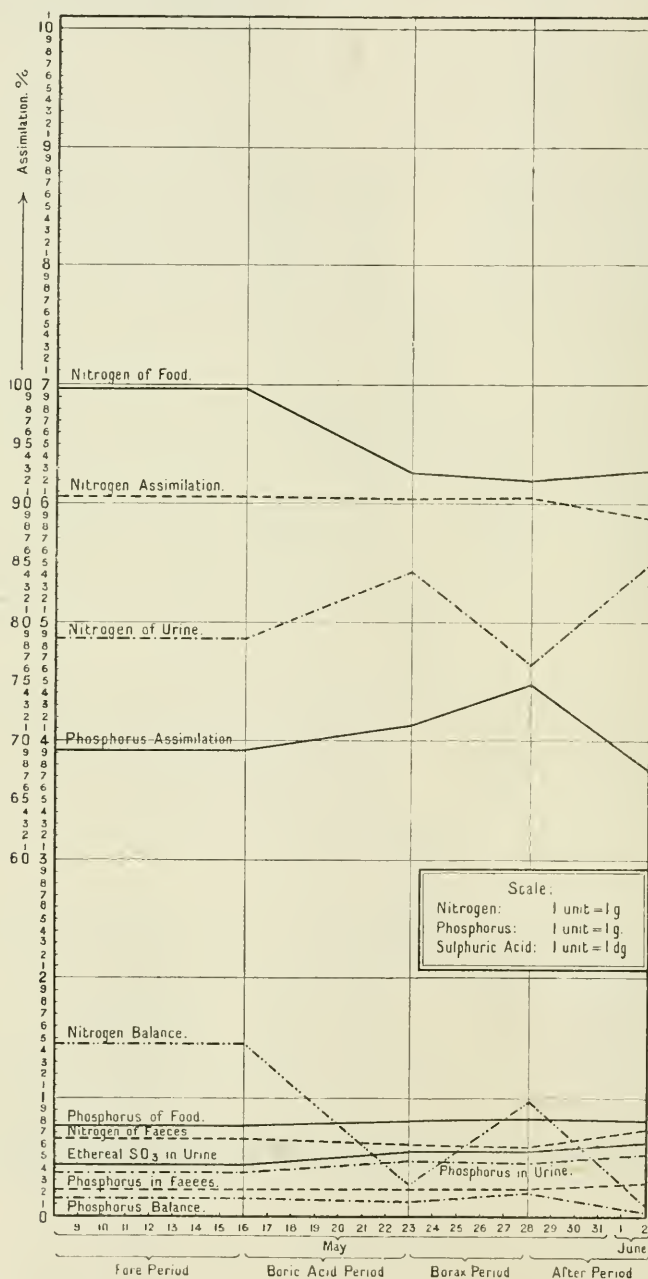
UPON THE GENERAL METABOLISM OF CHILD A, AGED $2\frac{1}{2}$ YEARS.

FAECES				Nitrogen of food	Balance	Body weight	PHOSPHORUS				FAT		
Moist g	Dry g	Water %	Nitro- gen g				Urine g	Faeces g	Food g	Balance g	Faeces g	Food g	Balance g
11	1.9	82.7	0.11	7.03	+2.40	15.28	0.2765	0.0455	0.76	+0.44	0.42	35.57	+35.15
88	14.5	83.5	0.86	7.17	+0.89		0.3571	0.3472	0.76	+0.06	3.23	35.57	+32.34
17	2.7	84.1	0.16	7.17	+1.86		0.3974	0.0647	0.76	+0.30	0.60	35.57	+34.97
114	18.0	76.0	0.06	7.17	+0.42		0.5126	0.4310	0.76	-0.18	4.01	34.96	+30.95
65	9.9	84.8	0.59	7.23	+1.51		0.5069	0.2371	0.76	-0.02	2.21	34.96	+32.75
60	7.6	87.3	0.49	7.23	+1.79		0.3471	0.1577	0.76	-0.26	1.85	34.96	+33.11
47	7.7	83.8	0.50	6.70	+1.99		0.3044	0.1598	0.81	+0.35	1.87	35.16	+33.29
116	21.4	81.4	1.39	6.28	+0.78	15.12	0.2613	0.4441	0.80	+0.09	5.19	37.46	+32.27
518	83.7		5.16	55.98	+11.64	-160g.	2.9633	1.8871	6.17	+1.34	19.38	284.21	+264.83
56	10.5	84.0	0.65	6.99	+1.45	-23g.	0.3704	0.2359	0.77	+0.17	2.42	35.53	+33.10
—	—	—	—	6.30	+0.39	15.12	0.4784	—	0.80	+0.32	—	37.46	+37.46
83	14.9	82.0	0.84	6.30	+0.30		0.3818	0.3144	0.80	+0.10	3.60	37.46	+33.86
35	8.4	76.0	0.47	6.30	-0.98		0.3636	0.1772	0.80	+0.26	2.03	37.46	+35.43
95	17.9	81.2	1.01	6.30	+0.40		0.5151	0.3777	0.80	+0.09	4.32	37.46	+33.14
60	13.4	77.7	0.76	6.30	-2.29		0.4784	—	0.80	+0.32	—	37.46	+37.46
109	22.2	79.6	1.26	6.30	-0.95	15.42	0.4600	0.2827	0.80	+0.06	3.24	37.46	+34.22
382	76.8		4.34	44.01	1.81	+300g.	3.1606	1.6204	5.61	+0.83	18.56	260.13	+241.57
54	10.9	80.0	0.62	6.29	+0.26	+43g.	0.4515	0.2315	0.80	+0.12	2.65	37.16	+34.51
10	1.5	85.0	0.10	6.21	+0.94	15.42	0.4424	0.0338	0.81	+0.33	0.31	35.17	+35.06
27	5.2	80.7	0.34	6.25	+0.91		0.4045	0.1172	0.81	+0.29	1.08	35.17	+34.29
74	12.5	83.1	0.81	6.41	+1.08		0.3792	0.2816	0.81	+0.15	2.59	41.42	+38.83
61	11.3	80.0	0.73	6.41	+1.56		0.5688	0.2546	0.81	-0.01	2.35	41.42	+39.07
72	15.0	79.2	0.97	5.71	+0.43	15.45	0.3413	0.3370	0.81	+0.13	3.12	41.42	+38.30
244	45.5		2.95	30.99	+4.92	+30g.	2.1362	1.0242	4.05	+0.89	9.45	195.00	+185.55
49	9.1	81.4	0.59	6.20	+0.98	+6g.	0.4272	0.2048	0.81	+0.18	1.89	39.00	+37.11
—	—	—	—	5.74	+0.73	15.45	0.4075	—	0.81	+0.40	—	41.68	+41.68
48	11.9	75.2	0.78	6.43	+0.70		0.3846	0.2812	0.81	+0.14	2.31	41.68	+39.37
80	16.4	79.5	1.07	6.44	-1.73		0.6544	0.3875	0.80	-0.24	3.18	41.68	+38.50
—	—	—	—	6.44	+1.58		0.4994	—	0.80	+0.30	—	41.68	+41.68
131	27.0	79.4	1.77	6.44	-0.88	15.45	0.5766	0.6380	0.80	-0.41	5.24	41.68	+36.44
259	55.3		3.62	31.49	+0.40	±0	2.5225	1.3067	4.02	+0.19	10.73	208.40	+197.67
52	11.0	78.6	0.72	6.29	0.08		0.5045	0.2613	0.80	+0.04	2.14	41.68	+39.53

The results expressed in the above table are graphically represented in the following curves:

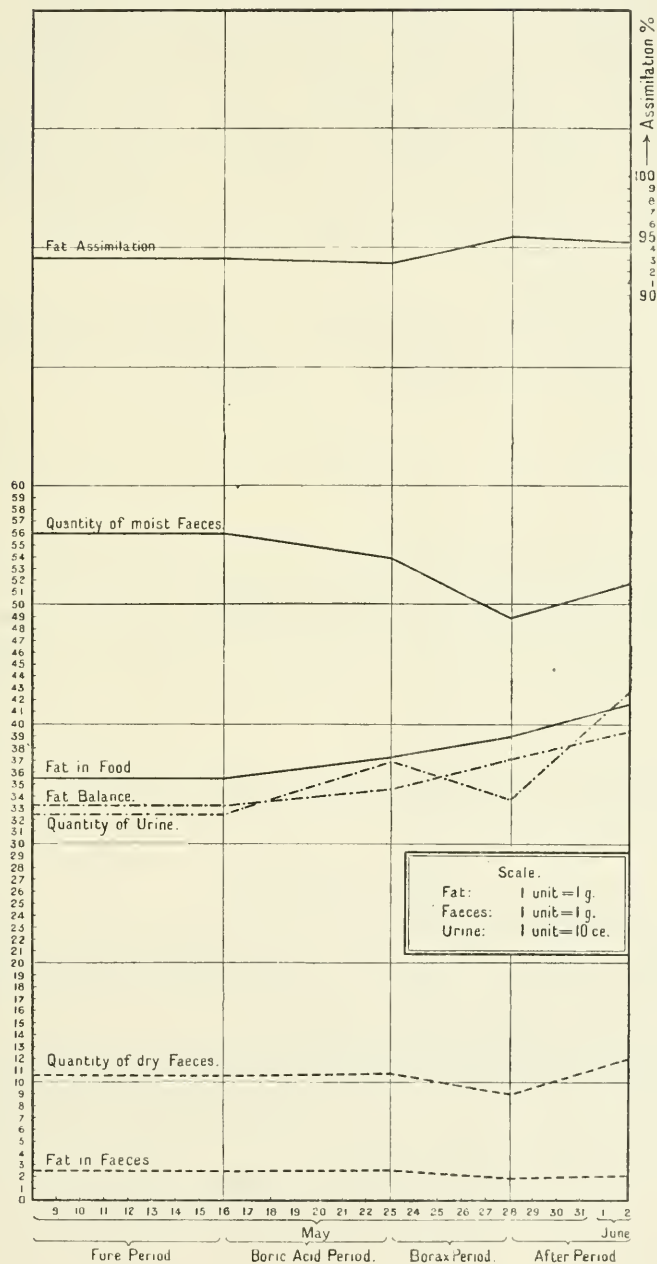
CURVE I,

showing the influence of boric acid and borax upon nitrogen and phosphorus metabolism, etc.



CURVE II,

showing the influence of boric acid and borax upon fat-assimilation and the quantity of faeces and urine.



OBSERVATION I. CHILD A.

The child was a healthy boy aged $2\frac{1}{2}$ years, weighed 15.3 kilos, and remained in good health throughout the whole observation. He consumed daily as follows, 200 g. of bread, 550 c.c. of milk, 20 g. of butter, 30 g. of meat, 50 g. of apple compote, 10 g. of sugar, 50 ccm. of water, 5 g. of toffee. This diet was very well taken and adhered to throughout the experiment. The whole observation extended over twenty-five days, eight days being taken as a fore period, and five days as an after period. The intermediate period of twelve days consisted of a boric acid period of seven days and a borax period of five days. The pure substances, boric acid or borax as the case may be, were added to 500 c.c. of the daily milk early in the morning and were administered as shown in the following table:

Boric Acid Period.

3 days : 0.5 g. per diem	= 1 in 1000 in Milk	= 1 in 1800 in total Food and Drink.
3 days : 0.66 g. ,,	= 1 in 760 ,,	= 1 in 1370 ,, ,, ,,
1 day : 1 g. ,,	= 1 in 500 ,,	= 1 in 900 ,, ,, ,,

Borax Period.

5 days : 1.5 g. per diem = 1 in 330 in Milk = 1 in 600 in total Food and Drink.

It may be noted here that the maximum medicinal dose for this child would be 0.2 g. of boric acid and 0.27 g. of borax, also that the quantities given are greatly in excess of those required for the preservation of milk¹.

The analytical results obtained throughout the observation are recorded in Table II. pp. 176-7.

Referring to the tables and curves relating to child A, we purpose classifying our remarks under the following headings :

Nitrogen Metabolism.

In the fore period the daily quantity of nitrogen taken in the food was 6.99 g., of which 0.65 g. were not assimilated, being lost with the faeces, corresponding to 9.3 %. The assimilation of nitrogen in the fore period amounted therefore to 90.70 %.

With the urine 4.88 g. of nitrogen were excreted, and if this amount be subtracted from the amount assimilated we obtain a daily balance of + 1.45 g. nitrogen.

¹ Vide Droop Richmond and Harrison, *Analyst*, 1900, p. 116.

To avoid repetition we give the results with regard to the nitrogen balance and assimilation during the different periods in tabular form :

—	Fore period	Boric acid period	Borax period	After period
Nitrogen in Food	6.99	6.29	6.20	6.29
„ „ Urine	4.88)	5.41)	4.62)	5.49)
„ „ Faeces	0.65]	0.62]	0.59]	0.72]
Balance	+ 1.45	+ 0.26	+ 0.98	+ 0.08
Assimilation $\frac{o}{o}$	90.70	90.14	90.48	88.56
Nitrogen $\frac{o}{o}$ in dry Faeces ...	6.0	5.7	6.5	6.5

From these results we are justified in concluding that neither boric acid nor borax exerts any influence upon the assimilation of proteids. The tendency of the body to reach nitrogenous equilibrium is clearly shown in the balance figures. From the amount of nitrogen excreted in the urine during the respective periods we may perhaps draw the conclusion that boric acid in this instance tends slightly to increase and borax slightly to inhibit proteid katabolism.

Phosphorus Metabolism.

The daily average quantity of phosphorus in the food during the fore period was 0.77 g., of which 0.2359 g. were lost, being excreted with the faeces. Phosphorus was therefore assimilated¹ to the extent of 69.36%. The relative excretion etc., of phosphorus in the four periods we give in tabular form.

—	Fore period	Boric acid period	Borax period	After period
Phosphorus in Food	0.77	0.80	0.81	0.80
„ „ Urine	0.3704)	0.4515)	0.4272)	0.5045)
„ „ Faeces	0.2359]	0.2315]	0.2048]	0.2613]
Balance	+ 0.17	+ 0.12	+ 0.18	+ 0.04
Assimilation $\frac{o}{o}$	69.36	71.06	74.82	67.34
Phosphorus $\frac{o}{o}$ in dry Faeces ...	2.2	2.1	2.2	2.4

These figures show that the phosphorus metabolism was not affected by boric acid and borax. The assimilation of phosphorus was if anything improved during the drug periods.

¹ The term assimilation in this sense is perhaps not strictly correct, we do not purpose however entering here into the actual source of the faecal phosphorus.

Fat Assimilation.

The daily quantity of fat in the food during the fore period was 35.53 g. The fat excreted with the faeces was 2.42 grammes. The assimilation therefore amounted to 93.19 %. These results and those of the following periods are recorded in tabular form as follows:

—					Fore period	Boric acid period	Borax period	After period
Fat in Food	35.53	37.16	39.00	41.68
„ Faeces	2.42	2.65	1.89	2.14
Fat balance	+ 33.10	+ 34.51	+ 37.11	+ 39.53
Assimilation %	93.19	92.87	95.19	94.87
Fat in dry Faeces %	23.0	24.3	20.8	19.5

It will be seen from these figures that the amount of fat retained by the body rose with the amount of fat in the food. If anything the assimilation of fat was increased during the borax period.

The chief remaining points of interest brought out by this observation are as follows¹:

The *quantity of urine* underwent slight variations during the drug periods in the direction of an increase. The increase was more marked during the boric acid period.

The *specific gravity* diminished as the volume increased.

The *reaction* alternating between acid and amphoteric (litmus) during the fore period remained constantly amphoteric during the boric acid period and constantly acid during the borax period.

The *quantity of faeces* remained practically unaltered with the exception of the borax period, in which the average daily quantity is slightly decreased.

The increase in *uric acid* is too slight to permit of any conclusions being drawn from it.

The quantity of total *sulphuric acid* increased slightly in the boric acid period, indicating with the slight increase of nitrogen in the urine a tendency to stimulate proteid katabolism.

The *etheral sulphates* were slightly increased during both periods to an equal extent. Intestinal putrefaction was therefore certainly not diminished by either substance, as was also shown by the comparative indoxyl-reactions.

¹ In these remarks throughout the entire paper we refer to the average daily excretion in question.

The boric acid could easily be demonstrated in the urine on the first day of its administration and disappeared completely in the course of the second day of the after period. These results show clearly that both boric acid and borax are rapidly eliminated from the body, and confirm the results of previous workers¹.

During the boric acid and borax periods the child gained in weight.

The results relevant to the observations made above are summarised in the following table:—

TABLE II A.

—	Nitrogen assimilation, %	% N. of dry faeces	Phosphorus assimilation, %	% P. of dry faeces	Fat assimilation, %	% Fat of dry faeces	A * B	N † SO ₃
Fore period ...	90·70	6·0	69·36	2·2	93·19	23·0	20·2	5·2
Boric acid period	90·14	5·7	71·06	2·1	92·87	24·3	17·6	5·2
Borax period ...	90·48	6·5	74·82	2·2	95·19	20·8	14·4	5·3
After period ...	88·56	6·5	67·34	2·4	94·87	19·5	17·1	5·1

$$\begin{aligned} * A &= \text{Inorganic SO}_3 \\ B &= \text{Ethereal SO}_3 \end{aligned}$$

$$\dagger \frac{N}{\text{SO}_3} = \frac{\text{Nitrogen of Urine}}{\text{SO}_3 \text{ of Urine}}.$$

OBSERVATION II. CHILD B.

The child was a healthy boy aged 5 years, weighing 18·5 kilos, and remained in good health during the whole observation. He consumed daily 250 g. of bread, 600 c.c. of milk, 20 g. of butter, 50 g. of meat, 50 g. of apple compote, 10 g. of sugar, 50 c.c. of water, and 5 g. of toffee. The whole observation lasted for twenty-two days. The fore period in this case lasted for 5 days, otherwise the arrangement and quantity of boric acid and borax given were the same as in Observation I. These substances were administered as shown in the following table:

Boric Acid Period.

3 days : 0·5 g. per diem = 1 in 1000 Milk, 1 in 2000 total Food and Drink.

3 days : 0·66 g. " = 1 in 760 " 1 in 1500 " " "

1 day : 1·0 g. " = 1 in 500 " 1 in 1000 " " "

Borax Period.

5 days : 1·5 g. " = 1 in 330 " 1 in 660 " " "

It may be noted here that the maximal medicinal dose for this child is in the case of boric acid 0·29 g., of borax 0·38 g., and that the quantities given, as in the last observation, are greatly in excess of those which would be required as a food preservative.

The analytical results obtained throughout this observation are recorded in the following table:—

¹ Chittenden and Gies (*loc. cit.*), where also references to former observers are given.

TABLE III.

SHOWING THE INFLUENCE OF BORIC ACID AND

PERIOD	—	Date	Dose g	URINE						
				Quantity c.c.	Reaction	Specific gravity	Total sulphuric acid g	Ethereal sulphuric acid g	Uric acid g	Nitrogen g
FORE PERIOD		12 V.		510	Acid	1·0194	1·4412	0·0899	0·1035	6·47
		13 "		395	"	1·0240	1·1762	0·0696	0·0802	6·59
		14 "		390	Amphoteric	1·0185	1·1020	0·0687	0·0792	4·64
		15 "		450	"	1·0228	1·2715	0·0793	0·0914	7·21
		16 "		470	"	1·0226	1·3281	0·0821	0·0954	7·41
	Total	5 days		2,215			6·3190	0·3896	0·4497	32·32
	Average	1 day		440		1·0214	1·2638	0·0779	0·0899	6·46
BORIC ACID PERIOD		17 V.	0·50	365	Acid	1·0275	1·1100	0·0744	0·0631	6·66
		18 "	0·50	400	"	1·0266	1·2165	0·0816	0·0692	6·94
		19 "	0·50	450	"	1·0225	1·3685	0·0918	0·0779	6·84
		20 "	0·66	305	"	1·0254	0·9276	0·0622	0·0528	5·26
		21 "	0·66	240	"	1·0175	0·7299	0·0489	0·0415	3·18
		22 "	0·66	600	"	1·0244	1·9629	0·1092	0·2250	10·27
		23 "	1·00	430	"	1·0280	1·4174	0·0783	0·1613	7·16
	Total	7 days	4·48	2,790			8·7328	0·5464	0·6908	46·31
	Average	1 day	0·64	398		1·0245	1·2475	0·0780	0·0987	6·61
BORAX PERIOD		24 V.	1·5	320	Acid	1·0228	1·0496	0·0717	0·0720	6·22
		25 "	1·5	410	"	1·0248	1·3448	0·0918	0·0923	7·23
		26 "	1·5	365	"	1·0255	1·1972	0·0818	0·0821	6·04
		27 "	1·5	430	"	1·0236	1·4104	0·0963	0·0968	6·87
		28 "	1·5	300	"	1·0290	0·9840	0·0672	0·0675	5·38
	Total	5 days	7·5	1,825			5·9860	0·4088	0·4107	31·74
	Average	1 day	1·5	365		1·0251	1·1972	0·0817	0·0821	6·35
AFTER PERIOD		29 V.		395	Acid	1·0195	1·2403	0·0672	0·1185	5·21
		30 "		335	"	1·0282	1·0519	0·0569	0·1005	6·41
		31 "		300	"	1·0300	0·9420	0·0510	0·0900	6·79
		1 "		425	"	1·0232	1·3345	0·0722	0·1275	7·08
		2 "		405	"	1·0215	1·2717	0·0688	0·1215	6·49
	Total	5 days		1,860			5·8404	0·3161	0·5580	31·98
	Average	1 day		372		1·0245	1·1681	0·0632	0·1116	6·39

TABLE III.

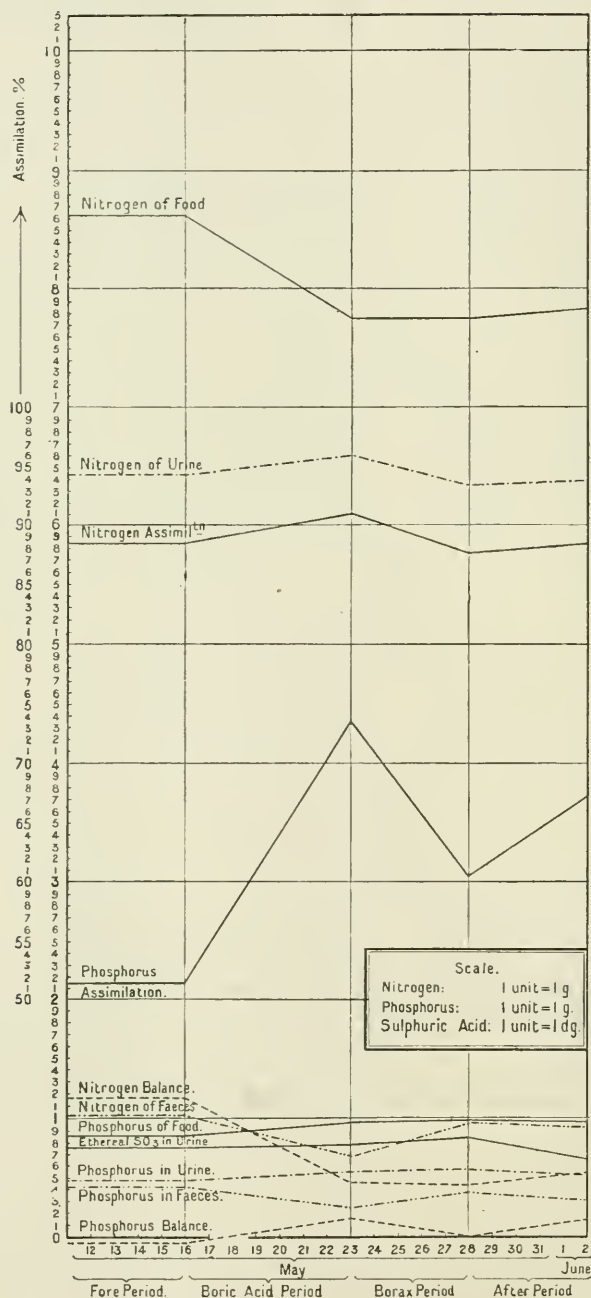
BORAX UPON THE GENERAL METABOLISM OF CHILD B.

FAECES				Nitro- gen of food	Balance	Body weight	PHOSPHORUS				FAT		
Moist	Dry	Water %	Nitro- gen				Urine	Faeces	Food	Balance	Faeces	Food	Balance
g	g		g	g	g	kg	g	g	g	g	g	g	g
—	—	—	—	8·99	+2·52	18·53	0·5712	—	0·85	+0·28	—	36·98	+36·98
110	27·2	75·3	1·84	9·05	+0·62		0·4527	0·7621	0·85	-0·36	4·68	36·98	+32·30
—	—	—	—	9·05	+4·41		0·4308	—	0·85	+0·42	—	36·98	+36·98
94	22·2	76·4	1·50	8·33	-0·38		0·5040	0·6220	0·85	-0·28	3·82	36·96	+33·14
107	25·2	76·5	1·71	7·78	-1·34	18·36	0·5264	0·7061	0·96	-0·27	4·33	41·05	+36·72
311	74·6		5·05	43·20	+5·83	-170g.	2·4851	2·0902	4·36	-0·21	12·83	188·95	+176·12
62	14·9	76·0	1·01	8·64	+1·17	-34g.	0·4970	0·4180	0·87	-0·04	2·56	37·59	+35·03
—	—	—	—	7·78	+1·12	18·36	0·4790	—	0·96	+0·48	—	41·05	+41·05
76	16·8	78·9	1·03	7·78	-0·19		0·5136	0·3861	0·96	+0·06	3·91	41·05	+37·14
—	—	—	—	7·78	+0·94		0·5778	—	0·96	+0·38	—	41·05	+41·05
96	20·5	78·6	1·27	7·78	+1·25		0·4002	0·4711	0·96	+0·09	4·77	41·05	+36·28
—	—	—	—	7·78	+4·60		0·3149	—	0·96	+0·65	—	41·05	+41·05
90	20·0	77·8	1·24	7·78	-3·73		0·8616	0·4596	0·96	-0·36	4·67	41·05	+36·38
94	21·1	77·6	1·31	7·72	-0·75	18·75	0·6174	0·4849	0·97	-0·13	4·91	37·50	+32·59
356	78·4		4·85	54·42	+3·26	+390g. Gain	3·7645	1·8017	6·73	+1·16	18·26	283·80	+265·54
51	11·2	77·8	0·69	7·77	+0·47	+56g.	0·5378	0·2574	0·96	+0·17	2·61	40·54	+37·93
—	—	—	—	7·72	+1·50	18·75	0·4932	0·4545	0·97	+0·02	—	37·50	+37·50
—	—	—	—	7·76	+0·53		0·6320	—	0·97	+0·34	—	37·50	+37·50
89	20·8	76·6	1·46	7·95	+0·45		0·5627	0·4545	0·97	-0·05	3·99	43·10	+39·11
55	12·9	76·5	0·91	7·95	+0·17		0·6628	0·2819	0·97	+0·03	2·47	43·10	+40·63
229	34·2	85·1	2·39	7·37	-0·40	18·78	0·4416	0·7472	0·97	-0·22	6·50	43·10	+36·60
373	67·9		4·76	38·75	+2·25	+30g. Gain	2·7923	1·9381	4·85	+0·12	12·96	204·30	+191·34
75	13·6	81·3	0·95	7·75	+0·45	+6g.	0·5584	0·3876	0·97	+0·02	2·59	40·87	+38·27
—	—	—	—	7·39	+2·18	18·78	0·5674	—	0·97	+0·40	—	43·36	+43·36
117	24·1		1·65	7·97	-0·09		0·4702	0·5644	0·97	-0·06	3·54	43·36	+39·82
132	13·0		0·89	7·97	+0·29		0·4211	0·3044	0·96	+0·23	1·91	43·27	+41·36
—	—	—	—	7·97	+0·89		0·5966	—	0·96	+0·36	—	43·27	+43·27
121	29·6		2·04	7·97	-0·56	18·81	0·5685	0·6932	0·96	-0·30	4·34	43·27	+38·93
370	66·7		4·58	39·27	+2·71	+30g. Gain	2·6238	1·5620	4·82	+0·64	9·79	216·53	+206·74
74	13·3	82·0	0·92	7·85	+0·54	+6g.	0·5247	0·3124	0·96	+0·13	1·96	43·30	+41·35

The results expressed in the above table are represented graphically in the following curves:

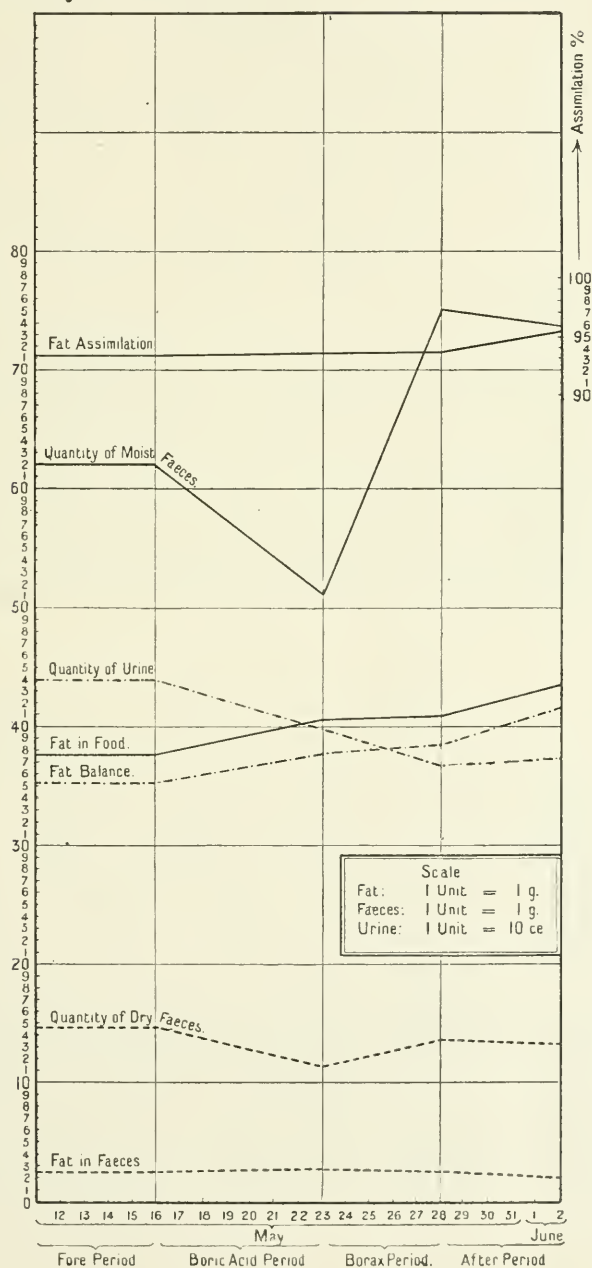
CURVE III.,

showing the influence of boric acid and borax upon nitrogen and phosphorus metabolism, etc.



CURVE IV.,

showing the influence of boric acid and borax upon fat metabolism and the quantity of faeces and urine.



Adopting the same method as in the previous observation we arrive at the following results:—

Nitrogen Metabolism.

—	Fore period	Boric acid period	Borax period	After period
Nitrogen in Food	8·64	7·77	7·75	7·85
„ „ Urine	6·46	6·61	6·35	6·39
„ „ Faeces	1·01	0·69	0·95	0·92
Balance	+1·17	+0·47	+0·45	+0·54
Assimilation %	88·31	91·12	87·74	88·28
Nitrogen % in dry Faeces ...	6·7	6·1	6·9	6·9

From these figures it will be seen that the nitrogen metabolism underwent no change. The assimilation of the nitrogenous food was improved during the boric acid period, and practically not affected during the borax period. The tendency of the body to reach equilibrium is clearly seen in the balance figures. The variation of the nitrogen in the urine during the respective periods is very small, but may be regarded as pointing to a slight stimulation of proteid katabolism during the boric acid period, and the reverse during the borax period.

Phosphorus Metabolism.

—	Fore period	Boric acid period	Borax period	After period
Phosphorus in Food	0·87	0·96	0·97	0·96
„ „ Urine	0·4970	0·5378	0·5584	0·5247
„ „ Faeces	0·4180	0·2574	0·3876	0·3124
Balance	-0·04	+0·17	+0·02	+0·13
Assimilation %	51·72	73·30	60·04	67·46
Phosphorus % in dry Faeces ...	2·9	2·3	2·8	2·3

From the above figures it will be seen that these drugs did not affect the phosphorus metabolism, but that the assimilation of phosphorus was rather improved by them, especially by boric acid. Taking into consideration the fact that the phosphorus in the food was increased during the drug periods, the slight increase of phosphorus in the urine cannot be regarded as pointing to an increased phosphorus katabolism.

Fat Assimilation.

—				Fore period	Boric acid period	Borax period	After period
Fat in Food	37.59	40.54	40.87	43.30
„ Faeces	2.56	2.61	2.59	1.96
Balance	+ 35.03	+ 37.93	+ 38.27	+ 41.35
Assimilation %	93.19	93.57	93.66	95.47
Fat in dry Faeces %	17.2	23.3	19.0	14.7

From the figures in this table it will be seen that the same remarks apply as in Observation I., viz. that boric acid and borax exerted no influence upon fat assimilation.

The remaining points to be considered may, as in the preceding observation, be divided as follows :

The *quantity of urine* was decidedly diminished during the borax period, to a less extent during the boric acid period. The specific gravity increased with the diminishing volume. The reaction of the urine kept constantly acid to litmus during both the boric acid and borax period. In the fore period it varied between amphoteric and acid.

Quantity of faeces. During the boric acid period the quantity of faeces was slightly decreased.

The *uric acid* variation is too slight to permit of any conclusion being drawn from it. The alteration in the quantity of total *sulphuric acid* during the respective periods was very slight, but in the same direction as that of the total nitrogen. The *etheral sulphates* underwent no change during the boric acid period, but increased slightly during the borax period. Neither substance exerted therefore any intestinal antiseptic action, the increase during the borax period is probably an alkali effect, the same having been observed in the case of other alkaline salts. Boric acid showed itself in the urine of the first day of its administration, and disappeared completely in the course of the second day of the after period.

The *body weight* increased during the boric acid and borax periods.

The results relevant to the observations made above are summarised in the following table :—

TABLE III A.

—	Nitrogen assimilation %	% N. of dry faeces	Phosphorus assimilation %	% P. of dry faeces	Fat assimilation %	% fat of dry faeces	A * B	N † SO ₃
Fore period ...	88.31	6.7	51.72	2.9	93.19	17.2	15.2	5.1
Boric acid period	91.12	6.1	73.30	2.3	93.57	23.3	15.0	5.3
Borax period ...	87.74	6.9	60.04	2.8	93.66	19.0	13.7	5.3
After period ...	88.28	6.9	67.46	2.3	95.47	14.7	17.5	5.5

* As in Table II A.

† As in Table II A.

OBSERVATION III. CHILD C.

The child was a delicate girl, aged four years, weighing 15.6 kilos. She was convalescent from pneumonia and compared with the other children not so well nourished or developed. She consumed daily 200 g. of bread, 550 c.c. of milk, 20 g. of butter, 30 g. of meat, 50 g. of apple compote, 10 g. of sugar, 50 c.c. of water, 5 g. of toffee.

The whole observation lasted for 22 days, of which five days were devoted to the fore period, seven days to boric acid, 5 days to borax, and 5 days to the after period.

The boric acid and borax were administered as shown in the following table :

Boric Acid Period.

3 days : 0.5 g. per diem = 1 in 1000 in Milk = 1 in 1800 in total Food and Drink.

3 days : 0.66 g. „ = 1 in 760 „ = 1 in 1370 „ „ „

1 day : 1.0 g. „ = 1 in 500 „ = 1 in 330 „ „ „

Borax Period.

5 days : 1.5g. „ = 1 in 330 „ = 1 in 600 „ „ „

It may be noted that the maximum medicinal dose for this child is in the case of boric acid 0.24 g., of borax 0.33 g., and that the quantities given as in the former observations, are greatly in excess of those which would be required as a food preservative.

The analytical results obtained throughout this observation are recorded in Table IV. p. 192 :—

Adopting the same method as in the previous observations we arrive at the following results with regard to,

Nitrogen Metabolism.

—	Fore period	Boric acid period	Borax period	After period
Nitrogen in Food ...	6.87	6.29	6.22	6.32
„ „ Urine ...	5.52	5.01	4.70	5.20
„ „ Faeces ...	0.72	0.65	0.75	0.72
Balance ...	+0.62	+0.62	+0.77	+0.39
Assimilation % ...	89.52	89.66	87.94	88.61
Nitrogen in dry Faeces %	6.3	5.6	6.4	7.0

The assimilation of proteids was in this case not affected by boric acid, but slightly decreased by borax. The balance remained practically constant, being near the equilibrium.

In this case boric acid does not seem to have stimulated proteid katabolism, whilst borax showed its usual inhibitory tendency.

Phosphorus Metabolism.

—	Fore period	Boric acid period	Borax period	After period
Phosphorus in Food	0·78	0·80	0·81	0·80
„ „ Urine	0·4399 }	0·4186 }	0·4168 }	0·4619 }
„ „ Faeces	0·2772 }	0·2406 }	0·2655 }	0·2410 }
Balance	+0·06	+0·14	+0·13	+0·10
Assimilation %	64·46	70·00	67·23	69·88
Phosphorus in dry Faeces % ...	2·4	2·1	2·1	2·3

As in the former cases the phosphorus assimilation was improved, especially in the boric acid period. The katabolism of substances rich in phosphorus seemed to be slightly inhibited in both periods.

Fat Assimilation.

—	Fore period	Boric acid period	Borax period	After period
Fat in Food	35·50	37·16	39·00	41·68
„ „ Faeces	2·35	2·44	2·48	1·80
Balance	+33·14	+34·71	+36·51	+39·88
Assimilation %	93·38	93·43	93·64	95·68
Fat in dry Faeces %	20·6	20·8	21·2	17·8

As in the former cases the fat balance increased with the amount of fat ingested. The assimilation of fat was not affected.

The remaining points to be considered may as in the preceding observations be classified as follows:

The *quantity of urine* decreased during the boric acid and borax period to the same extent, the *specific gravity* increasing with the diminishing volume. The reaction varied between acid and amphoteric during the boric acid period, and remained acid throughout the borax period.

The *quantity of dry faeces* underwent no change during the boric acid and borax period.

TABLE IV.

SHOWING THE INFLUENCE OF BORIC ACID AND BORAX UPON

PERIOD	—	Date	Dose g	URINE						
				Quantity c.c.	Reaction	Specific gravity	Total sulphuric acid g	Ethereal sulphuric acid g	Uric acid g	Nitrogen g
FORE PERIOD		12 V.		445	Amphoteric	1·0200	1·1861	0·0719	0·1902	5·74
		13 "		400	"	1·0226	1·0671	0·0646	0·1710	5·72
		14 "		480	Acid	1·0200	1·2805	0·0776	0·2052	6·48
		15 "		350	"	1·0227	0·9337	0·0566	0·1496	5·09
		16 "		275	Amphoteric	1·0270	0·7336	0·0444	0·1176	4·59
	Total	5 days		1950			5·2010	0·1151	0·8336	27·62
	Average	1 day		390		1·0224	1·0402	0·0630	0·1667	5·52
BORIC ACID PERIOD		17 V.	0·50	405	Acid	1·0200	1·2422	0·0818	0·1124	5·18
		18 "	0·50	320	"	1·0285	0·9816	0·0646	0·0888	5·93
		19 "	0·50	330	"	1·0269	0·8589	0·0667	0·0916	5·46
		20 "	0·66	260	"	1·0275	0·9662	0·0525	0·0722	4·75
		21 "	0·66	310	Amphoteric	1·0260	0·7208	0·0626	0·0860	5·40
		22 "	0·66	250	"	1·0295	0·8580	0·0590	0·1444	4·91
		23 "	1·00	200	Acid	1·0255	0·6864	0·0472	0·1155	3·44
	Total	7 days	4·48	2075			6·3141	0·4344	0·7109	35·07
	Average	1 day	0·64	296		1·0262	0·9020	0·0620	0·1015	5·01
BORAX PERIOD		24 V.	1·5	355	Acid	1·0236	1·0438	0·0774	0·2103	5·33
		25 "	1·5	320	"	1·0215	0·9409	0·0698	0·1896	4·39
		26 "	1·5	280	"	1·0260	0·8229	0·0610	0·1659	4·73
		27 "	1·5	315	"	1·0247	0·9267	0·0686	0·1866	4·76
		28 "	1·5	235	"	1·0276	0·6909	0·0512	0·1392	4·29
	Total	5 days	7·5	1505			4·4252	0·3280	0·8916	23·50
	Average	1 day	1·5	301		1·0247	0·8850	0·0656	0·1783	4·70
AFTER PERIOD		29 V.		410	Acid	1·0193	1·1572	0·0683	0·0738	5·51
		30 "		365	"	1·0236	1·0302	0·0608	0·0657	5·76
		31 "		270	"	1·0227	0·7620	0·0450	0·0486	4·53
		1 VI.		415	"	1·0187	1·1713	0·0691	0·0747	5·11
		2 "		295	"	1·0270	0·8326	0·0492	0·0531	5·12
	Total	5 days		1755			4·9533	0·2924	0·3159	26·03
	Average	1 day		351		1·0222	0·9906	0·0585	0·0632	5·20

TABLE IV.

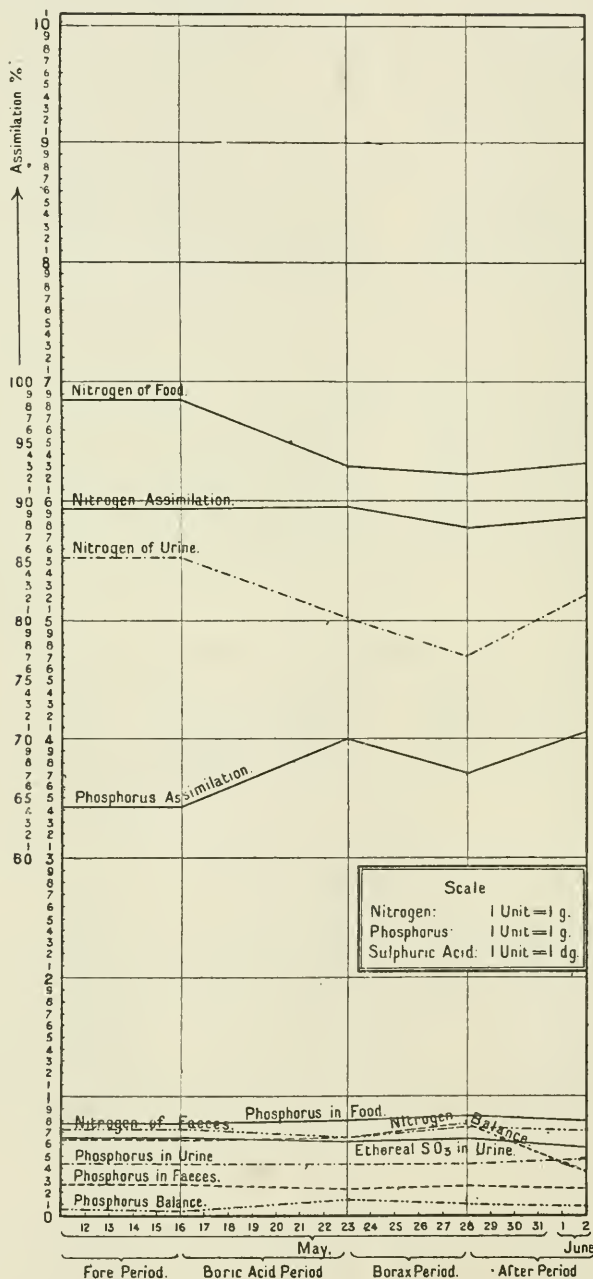
THE GENERAL METABOLISM OF THE INVALID CHILD C.

FAECES				Nitro- gen of food	Balance	Body weight	PHOSPHORUS				FAT		
Moist	Dry	Water %	Nitro- gen				Urine	Faeces	Food	Balance	Faeces	Food	Balance
g	g		g	g	g	kg	g	g	g	g	g	g	g
26	7.3	71.2	0.46	7.17	+0.91	15.62	0.5020	0.1785	0.76	+0.09	1.50	34.96	+33.46
—	—	—	—	7.23	+1.59	—	0.4512	—	0.76	+0.31	—	34.96	+34.96
69	15.6	77.4	0.99	7.23	-0.24	—	0.5414	0.3814	0.76	-0.16	3.21	34.96	+31.75
48	9.6	80.0	0.61	6.46	+0.76	—	0.3948	0.2347	0.81	+0.18	1.97	35.16	+33.19
109	24.8	77.2	1.57	6.28	+0.12	—	0.3102	0.5915	0.80	-0.10	5.10	37.46	+32.36
252	57.3	—	3.63	34.37	+3.12	15.62	2.1996	1.3861	3.89	+0.32	11.78	177.50	+165.72
50	11.4	77.3	0.72	6.87	+0.62	±0	0.4399	0.2772	0.78	+0.06	2.35	35.50	+33.14
—	—	—	—	6.30	+1.12	15.62	0.5464	—	0.80	+0.25	—	37.46	+37.46
165	18.8	88.6	0.94	6.30	-0.57	—	0.4317	0.4019	0.80	-0.03	3.55	37.46	+33.91
127	11.5	90.9	0.58	6.30	+0.26	—	0.4453	0.2458	0.80	+0.11	2.17	37.46	+35.29
9	1.6	82.2	0.08	6.30	+1.47	—	0.4857	0.0342	0.80	+0.28	0.30	37.46	+37.16
112	16.5	85.3	0.82	6.30	+0.08	—	0.4182	0.3527	0.80	+0.03	3.11	37.46	+34.55
68	12.7	81.3	0.82	6.30	+0.57	—	0.3350	0.2478	0.80	+0.22	3.05	37.46	+34.41
104	20.6	80.2	1.32	6.21	+1.45	15.84	0.2680	0.4019	0.81	+0.14	4.94	35.37	+30.43
585	81.7	—	4.56	44.01	+4.38	+220 Gain	2.9303	1.6843	5.61	+1.00	17.12	260.13	+243.01
83	11.7	86.0	0.65	6.29	+0.62	+31 g.	0.4186	0.2406	0.80	+0.14	2.44	37.16	+34.71
—	—	—	—	6.21	+0.88	15.84	0.4916	—	0.81	+0.32	—	35.37	+35.37
57	13.7	76.0	0.87	6.25	+0.99	—	0.4431	0.3098	0.81	+0.06	2.90	35.37	+32.47
48	10.6	77.9	0.67	6.41	+1.01	—	0.3878	0.2397	0.81	+0.18	2.25	41.42	+39.17
64	12.8	80.0	0.82	6.41	+0.83	—	0.4362	0.2895	0.81	+0.09	2.71	41.42	+38.71
141	21.6	84.7	1.39	5.84	+0.16	16.06	0.3254	0.4885	0.81	-0.01	4.58	41.42	+36.84
310	58.7	—	3.75	31.12	+3.87	+220 Gain	2.0841	1.3275	4.05	+0.64	12.44	195.00	+182.56
62	11.7	81.1	0.75	6.22	+0.77	+44 g.	0.4168	0.2655	0.81	+0.13	2.48	39.00	+36.51
—	—	—	—	5.85	+0.34	16.06	0.5406	—	0.81	+0.27	—	41.68	+41.68
58	11.0	81.0	0.79	6.43	-0.12	—	0.4814	0.2625	0.81	+0.07	1.96	41.68	+39.72
90	14.6	83.0	1.04	6.44	+0.87	—	0.3502	0.3484	0.80	+0.10	2.61	41.68	+39.07
61	10.0	83.6	0.72	6.44	+0.61	—	0.5484	0.2386	0.80	+0.01	1.78	41.68	+39.90
95	14.9	84.3	1.07	6.44	+0.25	16.00	0.3891	0.3555	0.80	+0.06	2.65	41.68	+39.90
304	50.5	—	3.62	31.60	+1.95	-60 Loss	2.3097	1.2050	4.02	+0.51	9.00	208.40	+199.40
61	10.1	83.4	0.72	6.32	+0.39	-12 g.	0.4619	0.2410	0.80	+0.10	1.80	41.68	+39.88

The results expressed in the above table are represented graphically in the following curves:

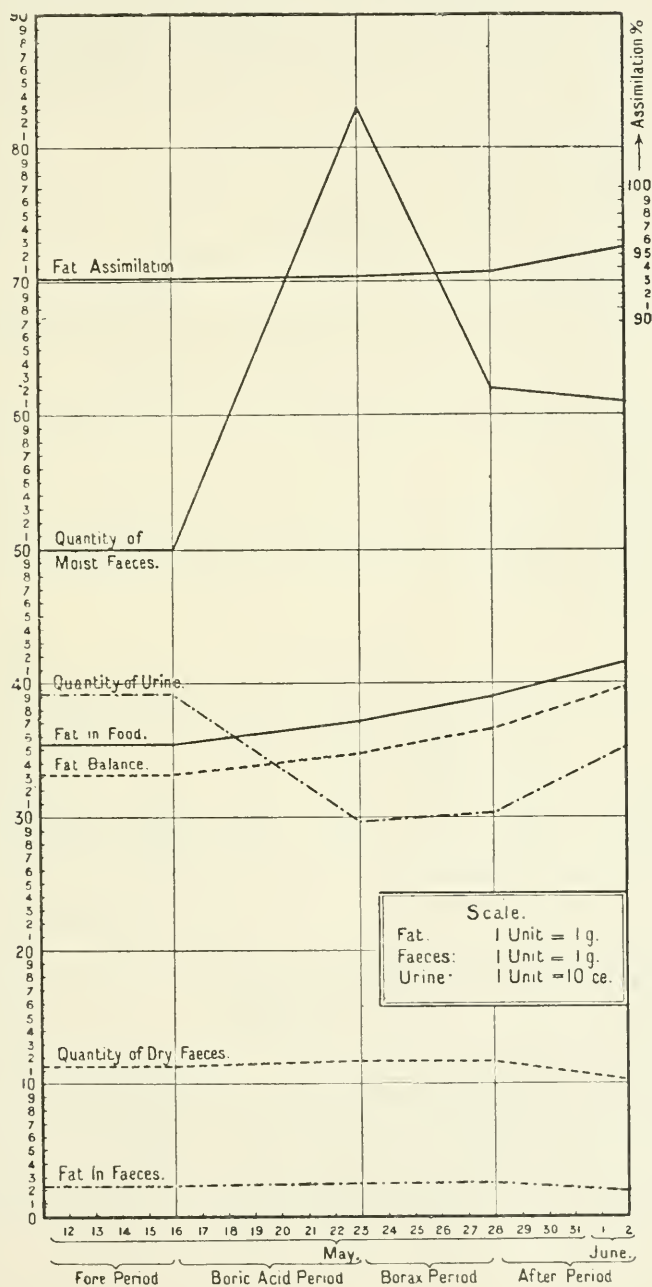
CURVE V.,

showing the influence of boric acid and borax upon the nitrogen metabolism of an invalid child.



CURVE VI.,

showing the influence of boric acid and borax upon the fat metabolism and the quantity of faeces and urine of an invalid child.



The *uric acid* excretion decreased somewhat during the boric acid period along with the decrease of nitrogen in food and the decreased nitrogen excretion in urine. During the borax period, however, we observed an increase in the amount of uric acid excreted, although the total nitrogen in the food and urine diminished. This seems to point to a specific uric acid solvent effect on the part of the borax and not to an increased uric acid formation, as in the after period the uric acid sank considerably below the fore period level.

The somewhat decreased quantity of *total sulphuric acid* excreted during the borax period, confirms the conclusion drawn from the decreased nitrogen and phosphorus, namely, that borax tends to slightly inhibit katabolism.

As in the former case, neither substance exerted an intestinal anti-septic action, borax probably by virtue of its alkalinity tending to increase the amount of ethereal sulphates eliminated.

Boric acid could be demonstrated in the urine in the first day of its administration, and was completely absent on the second day of the after period.

The body weight increased during both boric acid and borax period.

The results relevant to the observations made above are summarised in the following table :

TABLE IV A.

—	Nitrogen assimilation %	% N. of dry faeces	Phosphorus assimilation %	% P. of dry faeces	Fat assimilation %	% fat of dry faeces	A * B	N † SO ₃
Fore period ...	89.52	6.3	64.46	2.4	93.38	20.6	15.5	5.3
Boric acid period	89.66	5.6	70.00	2.1	93.43	20.8	13.5	5.5
Borax period ...	87.94	6.4	67.23	2.1	93.64	21.2	12.5	5.3
After period ...	88.61	7.0	69.88	2.3	95.68	17.8	15.9	5.2

* As in Table II A.

† As in Table II A.

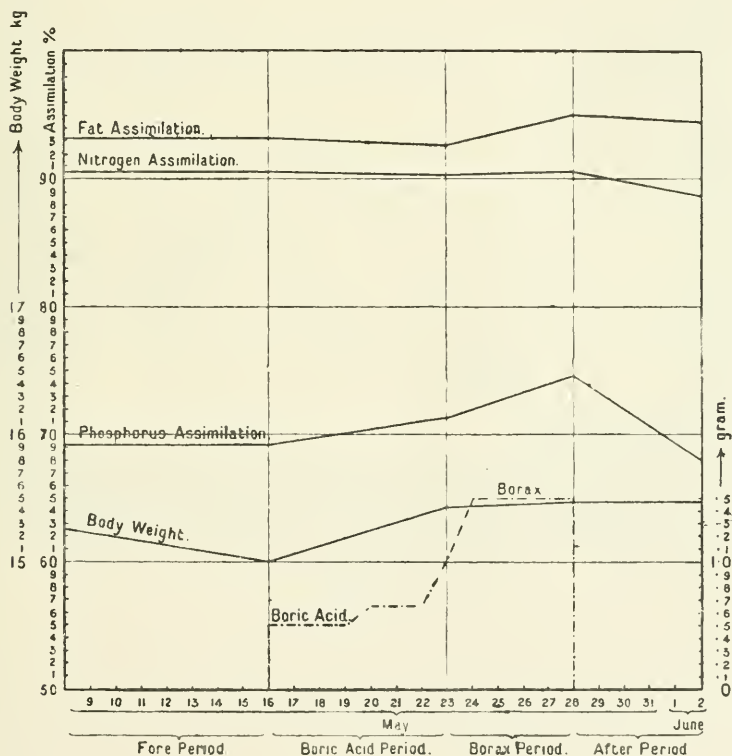
In all the three observations we estimated the amount of *lecithin* in the faeces during the normal and drug periods. The result of these investigations together with others will form the subject of a future paper, and we will restrict ourselves here to the simple statement that the excretion of lecithin with the faeces was diminished in each case during the borax periods. This observation, together with the fact of the improved phosphorus assimilation seems to point to a stimulating effect of this drug upon the pancreatic digestion, thus corroborating

in vivo what has already been shown *in vitro* (compare Chittenden *loc. cit.*).

Before proceeding to draw our general conclusions we give for the sake of reference, in our diagram, the result of the three observations expressed graphically, in so far as regards the influence of boric acid and borax upon nitrogen, phosphorus, and fat assimilation and body-weight.

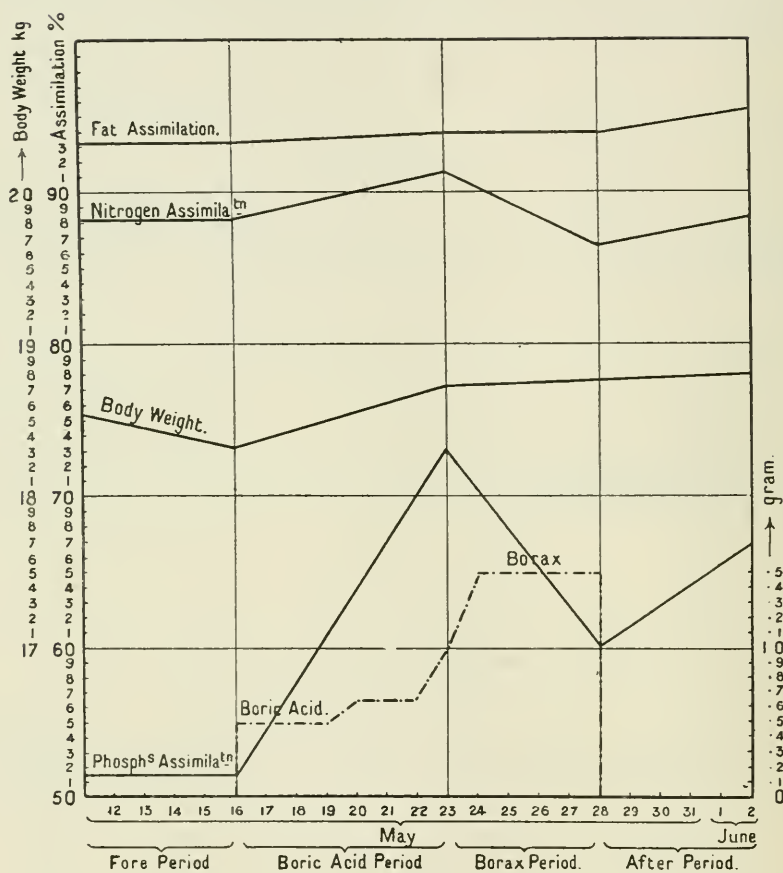
CURVE VII.,

showing the influence of boric acid and borax upon the body weight, and upon the nitrogen, phosphorus, and fat assimilation of these children.



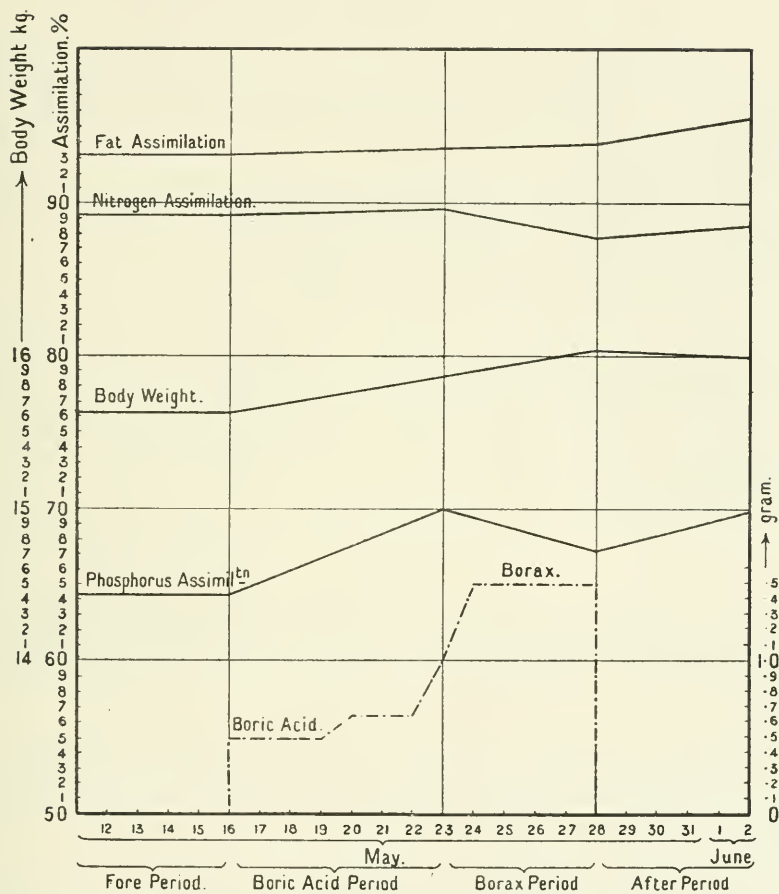
Child A.

CURVE VII.



Child B.

CURVE VII.



Child C.

GENERAL CONCLUSIONS.

Boric Acid.

(1) Small doses up to 1 gramme per diem, continued for some time, exert in healthy or delicate children no influence upon proteid metabolism. The assimilation of the proteid food was improved in one healthy child (B).

(2) The phosphorus metabolism was unaffected in all cases. The assimilation of phosphorus was in all cases improved.

(3) The assimilation of fat was not affected.

(4) The body weight increased in all cases.

(5) The quantity of dry faeces was not affected. Their nitrogen and phosphorus percentage was slightly decreased.

(6) No inhibitory effect upon intestinal putrefaction could be demonstrated.

Borax.

(1) Continued doses of 1.5 g. have no influence in healthy or delicate children upon proteid metabolism. The proteid assimilation was unaffected in healthy children, slightly depressed in the delicate child.

(2) The phosphorus metabolism was not affected in healthy or delicate children. The assimilation of phosphorus was improved in all cases, the improvement being least marked in the case of the delicate child.

(3) The fat assimilation was improved in the case of one healthy child, and unaffected in the case of the others.

(4) The body weight was increased in all cases; the increase was most marked in the case of the delicate child.

(5) The weight of dry faeces and their nitrogen and phosphorus percentage remained unaltered.

(6) Borax tended rather to increase intestinal putrefaction.

Boric Acid and Borax.

(1) Both boric acid and borax were quickly eliminated, no cumulative action being therefore probable.

(2) Neither boric acid nor borax in any way affected the general health and well-being of the children.

If we compare these results with those obtained in the only previous complete observation made by Forster (*loc. cit.*) on the action of boric acid upon the general metabolism of one adult man, we find that they are only in accord in so far as in neither could any material effect upon the general health and metabolism be observed. In none of our three cases, however, could we confirm Forster's single observation that boric acid caused an increase in the quantity of faeces and in their nitrogen and phosphorus percentage. Further in contradistinction to Forster we were unable to find that boric acid exerted any inhibitory effect on intestinal putrefactive action.

If on the other hand we compare our results upon children with those obtained by Chittenden and Gies (*loc. cit.*) with similar doses of these substances upon the metabolism of dogs, it will be seen that in the essential points they agree.

KING'S COLLEGE, LONDON.

Jan. 1901.

A COMPARATIVE STUDY OF VARIETIES OF *B. COLI* ISOLATED FROM "TYPHOID" AND NORMAL DEJECTA.

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THE want of success which has so persistently attended the efforts of most bacteriologists to isolate the *B. typhosus* from water supplies suspected to have caused enteric fever, suggested a study of the varieties of *B. coli* which are associated with the *B. typhosus* in the dejecta of patients suffering from enteric fever. It was hoped that the organisms in question might show cultural characteristics or reactions to specific sera, which would enable them to be distinguished from the varieties of *B. coli* present in the dejecta of healthy people; so that even if the *B. typhosus* were not detected, the presence of these special organisms might afford reasonable grounds for the belief that the water under examination had been fouled by the specific dejecta of cases of enteric fever. With this object in view 150 organisms have been examined; of these 80 were isolated from the stools of cases of enteric fever and 70 from the stools of healthy men. The enteric fever cases were five in number, one being a severe relapse, and the other four severe cases which terminated fatally. The stools were obtained during the third and fourth weeks of the disease and also, in the fatal cases, from the intestines after death had occurred. In order to isolate the organisms, one c.c. of each of the liquid stools was diluted 1—10,000 and 1—100,000 with distilled water, and $\frac{1}{10}$ c.c., $\frac{1}{4}$ c.c., and $\frac{1}{2}$ c.c. of these dilutions were plated out in gelatine. This method was not very satisfactory as the plates liquefied too rapidly. The use of carbolic acid might have helped to restrain the growth of liquefying organisms, but as this acid nearly always interferes with the perfect development of colonies, I thought it would be better to avoid its use if possible. After several trials, I found that the best results were obtained by taking a loopful of the liquid stool and then stroking it over the surface of a series of plates containing solidified gelatine. The first two or three plates usually liquefied, but the fourth, fifth and sixth plates showed discrete colonies which

developed satisfactorily. Typical colonies were fished and planted out on agar for further study.

Agglutination experiments. A twenty-four hours' agar growth of each organism was made into an emulsion with broth and then mixed with an equal bulk of horse's "anti-typhoid serum," also diluted with broth, so as to make final dilutions of the mixed emulsion and serum, of 1—50, 1—100, 1—200, 1—500 and 1—1000. At first the results were judged by the microscopic appearances in a hanging-drop at the end of two hours and the macroscopic appearances in capillary tubes at the end of twenty-four hours. Later, the hanging-drop method was omitted as a prolonged comparison of the two methods enabled the results obtained by the hanging-drop to be judged from the appearance of the capillary tube. The investigation of the 150 organisms extended over a period of four months, consequently it was necessary to employ different batches of horse's anti-typhoid serum. In order to make the results strictly comparable, the various specimens of serum were standardised week by week with two stock cultures of *B. typhosus* (K obtained from Dr Král's laboratory, and 13 P obtained from Prof. Wright of Netley). All through the experiments the sera used gave a complete agglutination in a dilution of 1—1000 and a marked reaction in a dilution of 1—10,000, with the two stock cultures.

TABLE A.

Varieties of B. coli isolated from typhoid stools.

+, = complete agglutination.

±, = marked agglutination.

-, = traces of agglutination.

0, = no agglutination.

	1—50	1—100	1—200	1—500	1—1000
1	+	+	±	0	0
2	+	—	0	0	0
3	+	—	0	0	0
4	+	+	±	0	0
5	0	0	0	0	0
6	+	+	±	0	0
7	0	0	0	0	0
8	0	0	0	0	0
9	0	0	0	0	0
10	0	0	0	0	0
11	0	0	0	0	0
12	+	+	±	0	0
13	0	0	0	0	0
14	0	0	0	0	0
15	+	+	±	0	0
16	+	+	±	0	0
17	0	0	0	0	0
18	0	0	0	0	0
19	0	0	0	0	0

TABLE A. (*cont.*)

	1-50	1-100	1-200	1-500	1-1000
20	0	0	0	0	0
21	0	0	0	0	0
22	0	0	0	0	0
23	0	0	0	0	0
24	0	0	0	0	0
25	0	0	0	0	0
26	0	0	0	0	0
27	+	+	±	-	0
28	+	+	±	-	0
29	+	+	±	-	0
30	+	+	±	-	0
31	+	+	±	-	0
32	+	+	±	-	0
33	-	0	0	0	0
34	-	0	0	0	0
35	-	0	0	0	0
36	+	+	±	0	0
37	+	+	±	0	0
38	+	±	±	0	0
39	+	+	-	0	0
40	+	+	±	0	0
41	+	+	±	0	0
42	+	+	±	0	0
43	+	+	±	0	0
44	+	+	±	0	0
45	0	0	0	0	0
46	0	0	0	0	0
47	0	0	0	0	0
48	0	0	0	0	0
49	0	0	0	0	0
50	+	-	0	0	0
51	+	±	0	0	0
52	+	±	±	0	0
53	+	±	±	0	0
54	+	±	±	0	0
55	+	±	±	0	0
56	+	±	-	0	0
57	+	+	±	0	0
58	+	+	+	-	0
59	+	±	0	0	0
60	+	+	+	0	0
61	+	+	+	0	0
62	+	+	+	+	+
63	+	+	+	+	-
64	+	+	+	+	-
65	+	+	+	+	±
66	+	+	+	+	±
67	+	+	+	+	±
68	+	+	+	-	0
69	+	+	+	+	-
70	0	0	0	0	0
71	0	0	0	0	0
72	0	0	0	0	0
73	0	0	0	0	0
74	0	0	0	0	0
75	0	0	0	0	0
76	0	0	0	0	0
77	0	0	0	0	0
78	0	0	0	0	0
79	0	0	0	0	0
80	0	0	0	0	0

Control tubes of the emulsions of all these organisms were kept under observation for 24 hours, but none of them showed the slightest trace of agglutination.

Note. Complete agglutination in a capillary tube means that all the bacilli were precipitated in a *firm globular mass* at the bottom of the tube. Marked agglutination means that the bacilli were precipitated in several firm globular masses scattered through the column, the portions of fluid between the masses being quite clear. Traces of agglutination means that a few globular masses were seen, but the remaining portions of the fluid were still opaque.

The results obtained with the 80 specimens of *B. coli* isolated from typhoid stools are shown in Table A. It will be seen that seven cultures (Nos. 62, 63, 64, 65, 66, and 69) were completely agglutinated by the typhoid serum diluted 1 in 500, two cultures (66 and 67) showed a marked reaction with the serum diluted 1 in 1000, and one culture (62) was completely agglutinated by the serum in this dilution. This last culture (62) was again tested at once with the serum in still higher dilutions, and it was found to be completely agglutinated when the serum was diluted 1—2500.

TABLE B.

Cultures of B. typhosus isolated from the spleens of fatal cases of enteric fever.

	1—50	1—100	1—200	1—500	1—1000	1—10,000
G	+	+	+	+	—	0
G* (1)	—	0	0	0	0	0
G* (2)	+	+	+	+	+	—
M	+	+	+	+	±	0
A	+	+	+	+	+	0
K	+	+	+	+	+	±
13 P	+	+	+	+	+	±

G* (1) tested immediately after isolation from the spleen.

G* (2) re-tested after being preserved for six months in milk.

In Table B are recorded the results obtained when the specimens of *B. typhosus* isolated from the spleens of the four cases of enteric fever, from which the stools were obtained, and the two stock cultures were tested with the same anti-typhoid serum. It will be observed that all

the specimens were completely agglutinated by the serum in a dilution of 1—500. Culture (G) *B. typhosus* and culture (62) *B. coli* were obtained from the same case, and it will be noticed that the *B. coli* was completely agglutinated by the serum in a higher dilution than was effective with the *B. typhosus*. If the serum used had been obtained from the patient it would have been easy to explain the result by assuming that the patient had suffered from a "mixed infection." The serum employed, however, was prepared by injecting a horse with *B. typhosus*, so a "secondary coli infection" is out of the question. It therefore appears that varieties of *B. coli* in typhoid stools may become agglutinated by a highly dilute anti-typhoid serum. That the reaction is only the result of environment, and is not truly specific as in the case of *B. typhosus*, is shown by the fact that when the cultures were removed from their associations and preserved for several months on agar, they gradually became incapable of reacting to a dilute serum. At the end of six months the results shown in Table C were obtained.

TABLE C.

Varieties of B. coli (from enteric stools) re-tested after being preserved for six months on agar.

	1—50	1—100	1—200	1—500	1—1000
62	±	±	0	0	0
63	±	0	0	0	0
64	+	+	0	0	0
65	+	+	0	0	0
66	+	±	0	0	0
67	+	±	0	0	0
68	—	0	0	0	0
69	+	+	±	—	0

The cultures of *B. typhosus* (G, M, A, K and 13 P), however, when tested at the end of six months showed no change in their reaction to the specific serum; the results recorded in Table B were again obtained. The *B. typhosus* culture G* was, however, peculiar; when first isolated from the spleen it showed only traces of agglutination with the specific serum diluted 1—50, six months later it was completely agglutinated by the serum diluted 1—1000. The cultural characteristics of this organism were carefully studied and compared with the other races of *B. typhosus*. The results are shown in the following Table D.

TABLE D. *Races of B. typhosus.*

	G	G*	A	M	K	13 P
Agar	Thin greyish-white growth	Thin greyish-white growth	Thin greyish-white growth	Thin greyish-white growth	Thin greyish-white growth	Thin greyish-white growth
Broth	Turbid, no film on the surface	Turbid, no film on the surface	Turbid, no film on the surface	Turbid, no film on the surface	Turbid, no film on the surface	Turbid, no film on the surface
Glucose or lactose-gelatin (shake)	No gas formation	No gas	No gas	No gas	No gas	No gas
Witte's peptone and salt solution, after 7 days at 37°C.	No indol	Traces of indol	No indol	No indol	Traces of indol	No indol
Potato	Moist, colourless growth	Colourless growth	Colourless growth	Colourless growth	Colourless growth	Colourless growth
Milk, 3 weeks at 37°C.	Unchanged	Unchanged	Unchanged	Unchanged	Unchanged	Unchanged
Litmus-whey, 7 days at 37°C., acidity equals	5.7 % $\frac{N}{10}$ alkali	5.1 % $\frac{N}{10}$ alkali	5.6 % $\frac{N}{10}$ alkali	5.4 % $\frac{N}{10}$ alkali	5.6 % $\frac{N}{10}$ alkali	5.5 % $\frac{N}{10}$ alkali
Proskauer & Capaldi's medium, No. I., 24 hours at 37°C.	No growth or change in reaction	Growth, but no change in reaction	No growth or change in reaction	No growth or change in reaction	No growth or change in reaction	Growth, but no change in reaction
Ditto, No. II., 24 hrs. at 37°C.	Strongly acid	Strongly acid	Strongly acid	Strongly acid	Strongly acid	Strongly acid
Gelatin plates	Colonies small and growth slow	Colonies small and growth slow	Colonies small and growth slow	Colonies small and growth slow	Colonies small and growth slow	Colonies small and growth slow
Motility (24 hours in broth)	Highly motile	Highly motile	Highly motile	Highly motile	Highly motile	Highly motile
Stained by Gram's method	No	No	No	No	No	No

TABLE E.
Varieties of B. coli isolated from typhoid stools.

	No. 62	No. 63	No. 64	No. 65	No. 66
Agar slope	Thick greyish-white growth	Thick greyish-white growth	Thick greyish-white growth	Thick greyish-white growth	Thick greyish-white growth
Broth	Turbid, no surface pellicle	Turbid, slight surface pellicle	Turbid, no surface pellicle	Turbid, no surface pellicle	Turbid, marked surface pellicle
Peptone (Witte) and salt solution, 7 days at 37° C.	No indol reaction	Traces of indol reaction	Marked indol reaction	Marked indol reaction	No indol reaction
Glucose and lactose-gelatin (shake)	Marked gas formation	Marked gas formation	Marked gas formation	Marked gas formation	Marked gas formation
Potato	Yellowish-brown growth	Thick yellowish-white growth	Thick yellowish-white growth	Thick yellowish-white growth	Thick yellowish-white growth
Milk (37° C.)	Coagulated in 7 days	Coagulated in 24 hours	Coagulated in 24 hours	Coagulated in 24 hours	Coagulated in 24 hours
Litmus-whey, 7 days at 37° C.	Acidity = 30 per cent. N alkali 10	Acidity = 37.8 per cent. N alkali 10	Acidity = 29 per cent. N alkali 10	Acidity = 34.5 per cent. N alkali 10	Acidity = 38 per cent. N alkali 10
Proskauer & Capaldi's No. 1. medium, 24 hours at 37° C.	Strongly acid	Strongly acid	Strongly acid	Strongly acid	Strongly acid
Ditto, No. 11., 24 hrs. at 37° C.	Faintly acid	Neutral	Faintly acid	Faintly alkaline	Neutral
Gelatin plates	Colonies grow rapidly, characteristic	Colonies grow rapidly, characteristic	Colonies grow rapidly, characteristic	Colonies grow rapidly, characteristic	Colonies grow rapidly, characteristic
Motility (24 hrs. in broth at 37° C.)	Not motile	Not motile	Not motile	Not motile	Not motile
Stained by Gram's method	No	No	No	No	No

TABLE E. (*cont.*)

	No. 67	No. 68	No. 69	No. 70
Agar slope	Thick greyish-white growth	Thick greyish-white growth	Thick greyish-white growth	Thick greyish-white growth
Broth	Turbid, slight surface pellicle	Turbid, slight surface pellicle	Turbid, no surface pellicle	Turbid, no surface pellicle
Peptone (Witte) and salt solution, 7 days at 37°C.	Traces of indol reaction	Marked indol reaction	Traces of indol reaction	Traces of indol reaction
Glucose and lactose-gelatin (shake)	Marked gas formation	Marked gas formation	Marked gas formation	Marked gas formation
Potato	Yellowish-white, rather dry growth	Thick yellowish-white growth	Thick yellow growth	Thick yellowish-brown growth
Milk (37°C.)	Coagulated in 24 hours	Coagulated in 24 hours	Coagulated in 48 hours	Unchanged after 19 days
Litmus-whey, 7 days at 37°C.	Acidity = 32.3 per cent. $\frac{N}{10}$ alkali	Acidity = 30 per cent. $\frac{N}{10}$ alkali	Acidity = 30 per cent. $\frac{N}{10}$ alkali	Neutral after 19 days
Proskauer and Capaldi, No. I., 24 hours at 37°C.	Strongly acid	Strongly acid	Strongly acid	Growth, but no change in reaction
Ditto, No. II., 24 hours at 37°C.	Neutral	Faintly acid	Neutral	Growth, but no change in reaction
Gelatin plates	Colonies grow rapidly, characteristic	Colonies grow rapidly, characteristic	Colonies grow rapidly, characteristic	Colonies grow rapidly, characteristic
Motility (24 hours in broth at 37°C.)	Not motile	Not motile	Not motile	Highly motile
Stained by Gram's method	No	No	No	No

It will be seen that culture G* agrees with the other members of the Table in most of the tests and must be pronounced an undoubted *B. typhosus*.

The cultural characteristics of the organisms (62), (63), (64), (65), (66), (67), (68), (69) and (70), were then carefully studied. It was thought that as most of them came within the "typhoid range" as regards agglutination, they might show an approximation to the cultural reactions of the *B. typhosus*. Table E gives the results obtained and shows that these cultures, with the exception of No. 70, presented all the chief typical reactions of *B. coli*. Cultures No. 62 and No. 66 only varied from the type by failing to produce indol. Culture No. 70, which was not agglutinated by the anti-typhoid serum, showed, however, considerable aberrations from the type; it was highly motile, did not produce acid in litmus-whey nor the characteristic reactions in Proskauer and Capaldi's media.

Forty-five other cultures (shown in Table A) were also examined as to the production of indol in peptone-and-salt solutions, acid in litmus-whey, souring of milk, and gas-formation in glucose-media. Twelve of these cultures failed to produce indol, and sour milk, and the amount of acid formed in litmus-whey was small, requiring only from 8 to 16 per cent. of decinormal alkali to neutralise it; the colonies, however, were typical and gas was produced in sugar-media. As regards the three tests usually considered typical of *B. coli*, viz., the production of indol, the formation of gas in sugar-media and the souring of milk, 64 per cent. of the colonies isolated gave all three reactions, 4·5 per cent. failed to produce indol, 4·5 per cent. failed to sour milk, and 27 per cent. gave only one reaction, viz., the production of gas in sugar-media, and were specially characterised by the small amount of acid formed in litmus-whey.

The results obtained show that *B. coli* from typhoid stools may be agglutinated by a highly dilute anti-typhoid serum, but this reaction is not necessarily nor usually associated with an approximation to the cultural characteristics of *B. typhosus*. The cultures which, according to their growths on the various media, approached most nearly to the *B. typhosus* were not agglutinated by a dilute anti-typhoid serum. On the other hand, culture G* shows that it is also possible for the *B. typhosus* to be present and yet show, when first isolated, no reaction to the specific serum.

Seventy colonies derived from the stools of healthy men were next investigated as to their cultural characteristics and reactions to anti-typhoid serum. The same procedure was followed as before.

As regards agglutination with anti-typhoid serum, Table F shows that not one of the varieties of *B. coli* derived from healthy stools was agglutinated by the serum in a dilution of 1—500, traces were seen but this reaction was so slight as to have no practical value. A complete reaction was only obtained twice with the serum diluted 1—100, and a marked reaction ten times with a dilution of 1—200. An anti-typhoid horse-serum certainly acts more strongly on *B. coli* than a normal horse-serum, but the same thing happens with water-organisms such as the *B. fluorescens liquefaciens* and *B. fluorescens putidus*. None of the anti-typhoid sera that I have examined when diluted 1—500 have ever completely agglutinated these organisms, no matter whether the experiment was performed in a capillary tube or a hanging-drop. Beco has isolated varieties of *B. coli*, from healthy stools, and *B. fluorescens liquefaciens* which were completely agglutinated by an anti-typhoid serum diluted 1—10,000. I have never obtained these results, though in one case I worked with a serum which when diluted 1—2,000,000, completely agglutinated the stock *B. typhosus* (13 P).

TABLE F.

Varieties of B. coli isolated from normal stools.

	1—50	1—100	1—200	1—500	1—1000
1	0	0	0	0	0
2	0	0	0	0	0
3	0	0	0	0	0
4	0	0	0	0	0
5	0	0	0	0	0
6	0	0	0	0	0
7	0	0	0	0	0
8	+	+	—	0	0
9	0	0	0	0	0
10	0	0	0	0	0
11	0	0	0	0	0
12	0	0	0	0	0
13	0	0	0	0	0
14	0	0	0	0	0
15	0	0	0	0	0
16	0	0	0	0	0
17	0	0	0	0	0
18	0	0	0	0	0
19	0	0	0	0	0
20	0	0	0	0	0
21	0	0	0	0	0
22	0	0	0	0	0
23	+	+	—	0	0
24	0	0	0	0	0
25	0	0	0	0	0
26	0	0	0	0	0
27	0	0	0	0	0

TABLE F. (cont.)

	1-50	1-100	1-200	1-500	1-1000
28	0	0	0	0	0
29	0	0	0	0	0
30	+	+	-	0	0
31	0	0	0	0	0
32	0	0	0	0	0
33	0	0	0	0	0
34	0	0	0	0	0
35	0	0	0	0	0
36	0	0	0	0	0
37	0	0	0	0	0
38	0	0	0	0	0
39	0	0	0	0	0
40	0	0	0	0	0
41	0	0	0	0	0
42	+	±	0	0	0
43	+	±	±	-	0
44	0	0	0	0	0
45	0	0	0	0	0
46	+	±	±	-	0
47	+	±	±	-	0
48	+	±	±	-	0
49	0	0	0	0	0
50	0	0	0	0	0
51	0	0	0	0	0
52	±	-	0	0	0
53	0	0	0	0	0
54	0	0	0	0	0
55	0	0	0	0	0
56	±	±	±	-	0
57	0	0	0	0	0
58	±	±	±	-	0
59	±	±	±	-	0
60	0	0	0	0	0
61	±	±	±	-	0
62	±	±	-	0	0
63	±	±	±	-	0
64	-	0	0	0	0
65	±	±	±	-	0
66	-	0	0	0	0
67	0	0	0	0	0
68	±	-	0	0	0
69	0	0	0	0	0
70	0	0	0	0	0

The cultural characteristics of the varieties of *B. coli* isolated from normal stools were briefly as follows:—48 per cent. gave the three typical reactions, 52 per cent. gave only two reactions, usually the souring of milk was absent. The acidity produced in litmus-whey varied from 27 to 47 per cent. of decinormal alkali. There were no constant characteristics by which the varieties of *B. coli* which reacted to the specific serum could be distinguished from those which showed no agglutination.

Conclusions.

(1) As regards the cultural characteristics on the various media employed, there appear to be no types of *B. coli* in typhoid stools which display sufficiently constant characters to enable them to be distinguished from the varieties of *B. coli* found in normal stools.

(2) As regards reaction to anti-typhoid horse-serum, the varieties of *B. coli* isolated from typhoid stools show much greater sensibility to agglutination than the varieties of *B. coli* isolated from healthy stools. Consequently, if varieties of *B. coli* isolated from a water-supply are found to be agglutinated with anti-typhoid horse-serum diluted 1—500, it would appear that there are reasonable grounds for the assumption that the water-supply in question has been fouled with the specific dejecta from cases of enteric fever.

A CONTRIBUTION TO THE AETIOLOGY OF EPIDEMIC CEREBROSPINAL MENINGITIS.

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THE following contribution to the aetiology of cerebrospinal meningitis is published with a view to calling attention to the possible part played by dust in the causation of this disease. The strong connection between dusty occupations and cases of cerebrospinal fever, as here detailed afford, it is believed, a *prima facie* presumption that dust may be one of the vehicles of the specific germ.

CEREBROSPINAL FEVER IN INDIA.

Though no less an authority than Hirsch⁽¹⁾ has stated that the Southern distribution of cerebrospinal meningitis (or cerebrospinal fever, as the disease is more conveniently called in India) is limited by the 30th degree of N. latitude, yet for the past 15 or 20 years the disease has been well known in India, the greater part of which lies well below the 30th parallel of latitude. The disease has also recently been recognised in Khartoum⁽²⁾, in the Ashanti field force⁽³⁾, and in the West Indies⁽⁴⁾, so that it is clear that its southern distribution can be limited by no such arbitrary line*.

In India the disease has chiefly been recognised among prisoners or similar collections of men, *e.g.* in the coolie emigration depots in

* EDITORIAL NOTE. Hirsch (1886, *Handb. d. histor.-geogr. Pathologie* Bd. III., p. 396) wrote "die Krankheit ist somit *bis jetzt* wesentlich auf gemässigte und subtropische Breiten beschränkt geblieben." Until the date of the appearance of his work there existed no *trustworthy* record of the disease further South than 30° N. L. both in the Eastern and Western Hemispheres. G. H. F. N.

Calcutta⁽³⁾, where labourers are collected prior to embarkation for the West Indies. In view of the latter fact it is strange that (as far as I can find out) the disease has only recently been discovered in emigrant ships trading with the West Indies⁽⁴⁾.

It is not however intended here to trace the history of the disease. So far as India is concerned I have done this in another place⁽⁵⁾.

The fact that this disease has chiefly been recognised among soldiers in barracks, and among prisoners, has led to an assumption that this incidence is in some way connected with defects in the hygiene of their surroundings. This may or may not be the case in other outbreaks, but in the three outbreaks, here detailed, the cases occurred at a time when (apart from this disease) the general health of the prisoners was remarkably good, the annual death-rate being about 13 per mille. It is possible that it is the greater care and attention to the diagnosis of disease and its registration which accounts for the seeming greater prevalence of this affection in such institutions.

THE BHAGALPUR PRISON OUTBREAKS.

The three separate epidemics or outbreaks which are here studied took place in the Central Prison, Bhagalpur, Bengal, during the four years 1897—1900.

The 47 cases here recorded were met with in irregular succession during four years, but they may, I think, be naturally divided into three separate outbreaks: viz. the first, extending from 11 January, 1897, till 18 April, 1897; the second from 17 October, 1897, till 20 April, 1898; and the third from 22 August, 1899, till the end of July, 1900.

Before January, 1897, a few cases of cerebrospinal meningitis had been noticed in this Jail: one case in 1889, one in 1890, six in 1891, and one in 1892. There were certainly no cases in 1893—1896. There was a six months' interval between the first and second outbreak, and an interval of sixteen months between the second and third.

First outbreak. The details of the first outbreak may be here summarised. There were nine cases from January till April 1897. All the patients were male Hindu adults, their ages varying from 24 to 45 years. Most of them had been confined in jail for at least three months (only one case, the seventh, had been there less than three months, viz. 36 days). The cases occurred in the following order of time, one case in January, another in February, two in March, and five in April. All died except the last case, which recovered after an acute attack.

These nine cases had been employed on the following forms of jail labour; six of the nine were employed in the jail garden, outside the walls, in gardening or in the

brickfield; of the three inside cases two were cook's attendants, and one was employed in sweeping roads inside the walls. The importance of these facts will be elucidated further on. Of these nine cases four came from Ward No. XV., and one case from each of Wards Nos. V., X., XII., XIII., XIV. (Total 9.)

Second outbreak. In this outbreak there were 14 cases, all in male Hindus, whose ages varied from 16 to 60 years. The period in jail before attack varied from 35 days to two years, but was usually about nine months. The infection was not brought in by the 35-day case, as it occurred eighth on the list. If we can accept so long an incubation period as 42 days, it is possible to say that the infection in this outbreak was introduced from outside by the boy who was attacked first, on October 17th. The next case, however, was in a man six months in jail, who was attacked two days after the first case.

The monthly distribution of these cases was as follows: October two cases, December one, January two, February two, April seven. From the local meteorological records it was found that in this, as in the first outbreak, strong dusty breezes had prevailed for a few days before the date of each case. The forms of labour furnishing cases were as follows: outside garden or the brickfield, nine cases; inside, rice-husking and wheat-grinding three cases, one was a cook's attendant, and one was from the indoor forms of labour in the blanket factory. The seven April cases occurred in true epidemic form on four successive days. As to the wards which produced cases, No. XV. had one; No. XI. two; the Juvenile Ward one; No. XII. two; No. XIII. one; No. X. one; No. VI. two; No. IV. one; No. II. one; No. XVI. two. (Total 14.)

Third outbreak. 24 cases occurred in the third outbreak, all amongst males, of whom 23 were Hindus and one a Mahomedan. Their ages varied between 19 and 40 years (average 30 years). The period in jail before attack varied from two months to seven years. The first case had been three months in jail, and the second over four months. The monthly distribution of the cases was as follows: August one, September one, October one, December one, March three, April thirteen, May two, July two. As in previous outbreaks the meteorological records show that strong winds had prevailed on or before the date of each attack. Cases came from gangs engaged on the following forms of labour: (a) gangs working in the garden or outside the jail walls, five; (b) wheat-grinding, four; (c) rice-husking, nine; (d) road-sweeping, three; (e) cooking, one; (f) convict messenger, one; (g) factory, a weaver. The cases in this outbreak came to hospital from a large number of wards, as follows: from No. III. one, from No. IV. three, No. IX. three, No. X. four, No. XI. one, No. XII. four, No. XIII. one, No. XIV. two, No. XV. five. In the third outbreak seven cases recovered and 17 died. (Total 24.)

Fatality. Of the 47 cases in the three outbreaks only fifteen recovered, a fatality of 68%. This is practically the same fatality as in the recent outbreak in Boston, Mass., where there were 111 cases with a mortality of 68½%. Rollet (1844) reported a rate of only 28% amongst his cases at Nancy, but the fatality has reached 75% in other epidemics (Bayonne, Aigues-Mortes). Hirsch states the fatality varies between 20 and 75%⁽⁶⁾.

Monthly Incidence of Cases. It is usually stated that this disease is chiefly met with in the spring and winter, this too has been our experience here, but most cases have occurred in the spring, as follows : January 3, February 3, March 5, April 25, May 2, June none, July 2, August 1, September 1, October 3, November none, December 2. In the following table the cases are grouped by seasons :

	Cases
Spring (March, April, May)	32
Summer (June, July, August)	3
Autumn (Sept., Oct., Nov.)	4
Winter (Dec., Jan., Feb.)	8
Total	47

In all three outbreaks most cases occurred in April, the month *par excellence* of hot and dusty wind-storms.

The Interval between Cases. In all recorded outbreaks the length of the interval between the successive cases has been very irregular. This has also been our experience, as the following figures show: In the *first outbreak* the first case occurred on January 11, 1897, and was followed by others at the following intervals: 24 days, 44 days, 9 days, 12 days, 4 days, 2 days (two cases), 2 days. In the *second outbreak* the second case followed the first after two days, then others with the following number of days between each: 48 days, 26 days, 22 days, 21 days, 13 days, 48 days (three cases), then another case after one day, then one day later three more cases. In the *third outbreak* (after 16 months free of cases), the first case was followed by the second in 29 days, then came cases with the following intervals between them: 28 days, 73 days, 66 days, 22 days, 1 day, 1 day, 1 day, 8 days, 4 days, then five cases on five successive days, then one 9 days later, then another after 22 days, then one after 49 days more, then after 10 days one. In each outbreak there was a run of cases in the middle period, *e.g.* in the first there were seven cases in four weeks, in the second seven cases in four days, and in the third sixteen cases in five weeks.

Age. Of the 47 cases only 2 were under 20 years of age. There were 8 from 21 to 25 years, 16 from 26 to 30 years, 11 from 31 to 40 years, 10 over 40 years of age. The juvenile prisoners suffered less proportionately to their numbers in the jail. The youngest case was aged 16, and the oldest 60 years.

Duration of Illness in Fatal Cases. Of the 32 fatal cases 18 were of the fulminant type, dying in three days or less. Of these 1 died in

about two hours. 6 in under 24 hours, and 4 in very little over the 24 hours. Twelve cases may be classed as acute, lasting from 3 to 15 days, and only 2 as chronic, lasting three or four weeks.

Mode of Onset. The sudden onset which is so characteristic of this disease was constantly noted in these cases. In only a few did the illness commence quietly, or with any noticeable premonitory symptoms. In almost every instance the patient had been at work as usual up to the evening before, or even on the morning of the day of attack. In one case the patient had been four days in the segregation-ward, on account of mumps, before the cerebral symptoms appeared. In only one case was the patient in bad health before the attack. As usual the disease chiefly attacked strong men.

Incubation Period. No evidence was obtained which throws any light on the length of the incubation period of this disease. If we suppose the germ to enter by means of the nasal cavities it probably does not take long to reach the brain meninges. The experience of the outbreak at Omdurman in 1898—99 showed that in some cases the incubation may be very short: as in two cases there, men sent to attend on other cases developed the disease within 52 and 76 hours respectively¹.

Prognosis. The prognosis is usually bad, the rate of fatality being very high. Relapses were not uncommon, and in some cases there were short intervals of apparently complete cessation of all activity of the disease. Lulls and temporary improvements were also not uncommon. For a favourable prognosis we have come to look upon the following as

¹ Since the above was written the question of the incubation period of Cerebrospinal Fever has arisen in connection with the epidemic in 1900 in the Calcutta Emigration Depots (see *Indian Med. Gazette*, vol. xxxvi. p. 78). An examination of the Jail Hospital Records has discovered the following facts about 6 cases in the third outbreak referred to in this paper: Case 1. An old man was discharged from hospital to work on rice-cleaning, and was admitted to hospital for cerebrospinal fever on July 27, or 6 days after going to work on this dusty form of labour.—2. Another man previously at work on the oil mill had his labour changed to rice-cleaning, on August 17, and after 3 days was admitted for the same disease.—3. Another man who had worked before in the factory was sent to work in the grain storeroom, and 8 days after came to hospital with this disease (April 23rd).—4. Another man was admitted 9 days after he was put into the outside garden gang.—5. Another man, a weaver, had his work changed on March 31st to be a storeroom messenger, and was attacked within 3 days.—6. Another man after having been in hospital for some trivial disease was sent to rice-husking on April 3rd, and was admitted to hospital for cerebrospinal fever on April 10th.

These cases also confirm a strong impression that such cases are apt to be met with a few days after a storm of wind and dust. It would probably be safe to put the incubation period of this disease at from 2 to 7 days.

important: a cleaning tongue, the disappearance of Kernig's symptom, and most favourable of all, a prolonged and quiet sleep. As an early aid to diagnosis Kernig's symptom is invaluable.

Association with Pneumonia. In only three cases in this series was there any association with pneumonia. In one case lobar pneumonia of the right base appeared towards the end of an acute case, in another a patch of central pneumonia was found, in a third a condition, which is noted as "resembling commencing pneumonia" is recorded. There was no special prevalence of pneumonia during any of the outbreaks.

Skin-eruptions. The only form of skin-eruption or rash observed in any of these cases was herpes. This was nearly always present, on the face or other parts of the body. It appeared at no regular period of the attack, and had no special prognostic significance. No form of petechial rash was observed.

Bacteriology of the Disease. It is now generally recognised that the microorganism of this disease is the *Diplococcus intracellularis meningitidis* of Weichselbaum. Other forms of meningitis are due to various organisms, but this *Diplococcus* is admitted to be the cause of the epidemic meningitis, which is called in the Nomenclature of Diseases of the Royal College of Physicians, London, "Cerebrospinal Fever."

For a full account of the history and the characteristics of this organism the reader is referred to the masterly monograph by Drs Councilman, Mallory and Wright, of Harvard University⁽⁶⁾. So far as I can learn the first described finding of the *Diplococcus* in India was in a case reported by myself in 1898. In the following year, in one of the cases in the second outbreak mentioned above, I sent specimens of the morbid material from the brain to Major F. J. Drury, M.B., I.M.S. &c., Professor of Pathology, Medical College, Calcutta, who reported the finding of a *Diplococcus*⁽⁷⁾ in them which strongly resembled the published accounts of the *Diplococcus* of Weichselbaum. More recently, in the case which died July 23rd, 1900, I also sent specimens to Captain Leonard Rogers, M.D., I.M.S., the then acting-Professor of Pathology at the Medical College, Calcutta, and he reported the finding of the same organism. Since then Dr Rogers has had an opportunity of examining five cases belonging to an epidemic of the disease at the emigration-depots in Calcutta, and found in all five the same organism⁽⁸⁾.

This then may be considered to establish the identity of the disease

in India with that known in other countries, a conclusion to which the whole history and symptoms of the disease strongly pointed.

Dust as a Vehicle of the Specific Germ. We now come to the main object of this paper, which is to point out the strong evidence which connects cases of this disease with dust, a connection pointed out to me by Captain C. R. Stevens, F.R.C.S., I.M.S., in conjunction with the cases in the third outbreak detailed above.

Germano has shown that the *Diplococcus intracellularis* can "resist desiccation, and preserve its vitality to the end of even ninety days," (this statement is quoted on the authority of the Report of the Sanitary Commissioner with the Government of India for 1898, p. 119)¹. The following facts therefore are of special interest in this connection. Dust, it is presumed, is only the vehicle for the specific germ, and the medium in which it can remain latent and potentially active, till some unknown favouring condition rouses it into activity (possibly warmth or overcrowding or both), or it may be that the dust only

¹ EDITORIAL NOTE. The *Diplococcus intracellularis* Weichselbaum, judged from its behaviour in cultures, has been generally regarded as incapable of leading a saprophytic existence, and it as a rule rapidly becomes attenuated when cultivated. There are but few observations mentioned in the literature regarding its resistance to desiccation. Jaeger (1894) is quoted by Germano (1897, *Zeitschr. f. Hyg. u. Infektionskr.* xxvi. p. 288—291) as having found the *Diplococcus* in a handkerchief six weeks after its being used by a patient suffering from cerebrospinal fever. Germano (*loc. cit.*) obtained his cultures from Jaeger. He made a concentrated suspension in bouillon of fresh agar cultures of the *Diplococcus*, dried it in Petri dishes over H_2SO_4 within 24 hours, broke up the dry substance with a glass rod and subsequently made agar plates with the material at various intervals of time, having previously mixed it with dust obtained from a room, with sand, earth (humus), marl, or brick-dust. Some of the material was kept moist, some of it air-dry, some of it quite dry over H_2SO_4 . The *Diplococci* in all samples survived 80—90 days; in certain experiments, especially those with room-dust, a decrease in the number of germs was noted. Germano concludes that the *Diplococcus* is one of the most resistant of the non-sporogenic bacteria, and that it may very well cause infection when floating in the air as dust. Germano says nothing about having tested the virulence of the *Diplococci* he subjected to desiccation, which is certainly to be regretted. Councilman, Mallory and Wright (1898, p. 78; see Dr Buchanan's reference No. 6) dried blood-serum cultures of the *Diplococcus* on paper (the kind of paper used is not stated) and kept the paper in Petri dishes. When kept at room-temperature and in the dark, the organisms, as tested by cultures, were alive after 24—60 hours' desiccation, but not after 72—96 hours. *Diplococci* dried on paper, and kept at 37·5° C. for 24 hours were dead. The difference in these results may be due to differences in the individual cultures used, the viability being known to vary greatly even in cultures derived from the same stock. Moreover Kamen (1898, *Centralbl. f. Bakteriöl.* xxiv. p. 555) believes that *Diplococci* cultivated for several generations outside the body possess greater viability than those derived directly from man. G. H. F. N.

acts indirectly by injuring the air passages and diminishing resistance.

Before, however, going on to show how the cases in the above three outbreaks are connected with dust, it is necessary to say something of the industries carried on in this prison.

The average number of prisoners varies from 1700 to 1800, the accommodation being for 1804. The chief industry of the prison is the manufacture of blankets for the army, some 700 prisoners being daily employed, indoors, on the various processes of weaving and spinning. Others are employed as work-overseers, tailors, carpenters, in carpet-making, and other minor industries. This accounts for some 1100 or so of the total population. All these industries are carried on *indoors*. The remainder of the population (excluding those under trial, in hospital, and females) are employed, either outside the walls of the prison in farming, gardening and in the brickfield, &c., or inside in workshops, in rice-husking, rice-cleaning, wheat- or maize-grinding, or as cooks or ration-storehouse attendants. Usually there is also a gang of old or weakly men employed as road-sweepers, and another gang is in charge of the latrines. These various employments afford work for the remaining six or seven hundred of the population.

One broad distinction may be drawn between the two classes of labour, the thousand or so men employed in the Manufacturing Department are for the most part of the day confined indoors, within the substantial Factory Buildings, and are but little exposed to dust, whereas the other 700, employed either outside the walls or on the various forms of grain-cleaning, are constantly and greatly exposed to dust, either that blown about by the wind, or that produced copiously in the processes of grain-husking, &c.

It has already been shown that cases of this disease occurred chiefly in the spring and dry weather months, especially in April, when dry, hot dust-storms are of daily occurrence, and blow steadily from 11 a.m. till 4 p.m. It is also a significant fact that out of the 47 cases here recorded no less than 44 came from what may be called "the dusty occupations," and only 3 from the indoor industries. It is clear therefore that the men employed on the dusty forms of labour were more exposed to the germ of the disease than the considerably larger body of men employed indoors.

The following table shows this in a graphic manner:

Table showing the influence of dusty and dust-free occupations upon the prevalence of cerebrospinal fever in Bhagalpur Central Jail.

OCCUPATIONS WITH EXPOSURE TO DUST		INDOOR OCCUPATIONS, WITHOUT EXPOSURE TO DUST	
	Cases		Cases
Public works and roof-repairing gangs	5	Factory Weaver	2
Gardening gangs	8	Factory Storeroom	1
Brickfield gangs	4		
Road-sweepers	2		
Limekiln	2		
Road-making	3		
(Food-preparation gangs)			
Cooks' attendant	4		
Wheat-grinding	6		
Pulse-cleaning	2		
Rice-husking	7		
Rice-cleaning	1		
Total	44		3

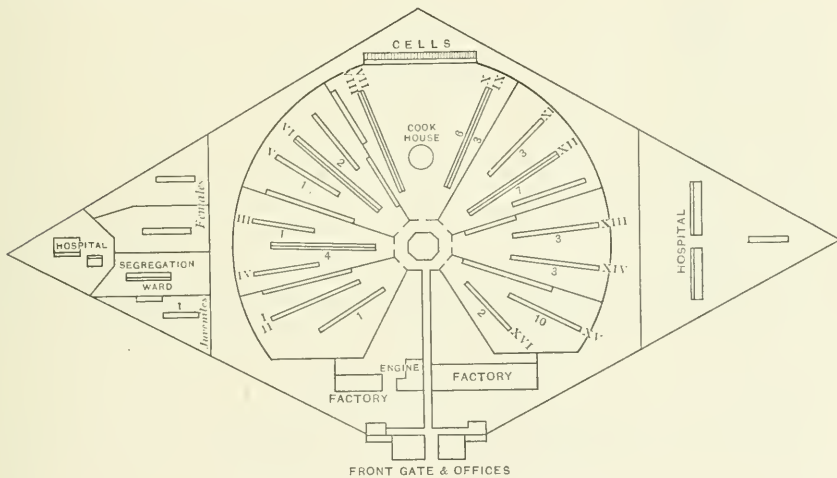
The influence of dust is also apparent from the following facts: the months from the middle of June till the middle of October make up the rainy season, when dust on the ground is at its minimum; now in these months there were in all only seven cases, and, what is important in view of the dust theory, is that every one of these seven cases came from forms of labour in which they were exposed to dust almost as much as they could be in the dry weather, viz., July, one case from rice-cleaning, and one from the wheat-grinding shed; August, one case, a cook's attendant; September, one case from the wheat-grinding shed; October, three cases, one each from the pulse-cleaning, wheat-grinding, and roof-repairing gangs.

I believe I am safe in claiming that the facts here presented form a strong case in favour of the presumption that dust is the vehicle of the germ of this disease. For anyone who knows India in the dry and hot weather it is not necessary to show how dust pervades everything and everybody in these seasons. It may also be stated that the men employed on the various forms of grain-cleaning emerge from their workshed as dusty as the proverbial miller.

The Source of the Infection. It is impossible to say whence the infection was first derived. When the first case occurred in these outbreaks there had not been a case inside the jail for four years, and

the disease was not recognised as existing among the outside population. The first man attacked had been one year in prison, so could not have brought the infection with him from outside; he was, however, employed in the garden just outside the jail walls. The second case did not follow till after three weeks, when a cook's attendant was attacked, who had been two months inside the jail, and never outside during that time.

In the second outbreak, six months after the last previous case, the first person attacked was a boy who had been six weeks in prison, and employed inside; the next case came two days later in the person of a man employed in repairing buildings outside the walls. In the third outbreak, which began sixteen months after the last previous case, the origin of the infection is equally unknown; the first case was in a cook, over three months in jail, the second case came a month later, then a third after another month, a fourth after two more months, then a run of 18 cases in six weeks.



PLAN OF CENTRAL JAIL, BHAGALPUR.

Roman Numbers I to XVI denote wards. Other Numbers denote cases of Cerebrospinal Fever.

It is possible of course that the disease *may* exist among the free population outside the jail. It has however never been recognised among them.

It has proved equally impossible to trace any connection between individual cases, they came at irregular intervals, from different forms

of labour and from different wards. Exposure to dust seems the only link which connects them.

The Question of Ward Infection. Out of the twenty-two barracks or wards in the Jail, cases came in the three outbreaks from all except six. Some wards however furnished very few cases. The following table summarizes the occurrence of cases in the different wards.

Table showing occurrence of cases of cerebrospinal fever in Bhagalpur Central Jail in the different Wards during three outbreaks.

No. of Ward	Number of cases occurring in each Ward during three outbreaks			Totals in each Ward
	I	II	III	
II	—	1	—	1
III	—	—	1	1
IV	—	1	3	4
V	1	—	—	1
VI	—	2	—	2
VII	—	—	—	—
VIII	—	—	—	—
IX	—	—	3	3
X	1	1	4	6
XI	—	2	1	3
XII	1	2	4	7
XIII	1	1	1	3
XIV	1	—	2	3
XV	4	1	5	10
XVI	—	2	—	2
Juveniles' Ward	—	1	—	1
Totals	9	14	24	47

Number of Wards affected.

Outbreak	I	6 wards
"	II	10 "
"	III	9 "

The ward which produced most cases in the first outbreak was No. XV. with 4 cases, all from the same outside gangs and all within eighteen days; this points, at least, to infection from the same source.

In the third outbreak in ward No. XV. four cases occurred (two from rice husking and two from outside gangs) within four days, two cases came from No. XII. on two successive days, and two from No. IX. within three days. These facts point to a common source of infection rather than a spread of the disease from case to case. It may be added

that ward No. XV., in which the greatest number of cases have occurred in the three outbreaks, is one of the old-fashioned tile-roofed wards, in which (from considerations of safe custody) only prisoners with short sentences (total or remaining) are confined, and such prisoners are usually employed either in the garden, or on non-skilled forms of labour as grain-cleaning &c.

Contagion. It may be said at once that no evidence of direct contagion from case to case was obtained in any of the above outbreaks; in no case were any of those in attendance on the sick attacked, though it was possible to keep many of them under observation for many months after their attendance.

The distribution as given above of cases from the different wards is, I think, to be explained by the fact that the prisoners who occupied these wards at night were also associated during the day on the forms of labour which specially seem to expose the workers to the infection. It is of course not impossible that the infection in some cases was contained in the dust in the roofs of such old barracks as No. XV. or No. XIII. The roofs of these old-fashioned wards are made of bamboo mats and tiles and certainly contain a large quantity of accumulated dust. Such a thing is not possible in the majority of the barracks, which are fine lofty new buildings with cemented floors and flat cemented roofs. That the dust accumulated in old roofs has aroused suspicion in others as being a possible nidus for the germ is clear from the following quotation from the account of the outbreak at Omdurman⁽²⁾: "Apparent infection through dust which clings to old mats, walls, roofs, etc.....so that when these are removed they become a source of infection *en route* and in their new position."

Overcrowding. This factor is usually invoked in every outbreak of cerebrospinal fever, though, as a matter of fact, soldiers and prisoners are now better housed than members of the general public of the same social class. In this prison the capacity of each ward is calculated so as to give 36 sq. feet of superficial space per man, and in the new barracks this is increased to 40 sq. feet. The area of ventilation per man is no less than from 12 to 14 sq. feet, and in the warm weather, with the grated doors and windows open, the ventilation is ample and practically unlimited. Nevertheless though overcrowding is clearly recognised as a defect, and stringent rules are laid down for its avoidance, anyone acquainted with prison management must be aware that at certain times some degree of overcrowding is unavoidable. An examination of the "lock-up register," which daily records the numbers

confined in each ward, shows that on the majority of occasions when cases of cerebrospinal fever occurred in the wards, there was either very slight overcrowding, or at least the ward was full up to its registered capacity, in only 13 cases out of 47 was the ward less than full. We may therefore look upon overcrowding as having some influence, probably of a predisposing nature, on outbreaks of this disease. In no instance, however, was there any marked overcrowding, or for any prolonged period.

For the working out of the dust theory, in connection with the cases in the third outbreak, I am indebted to Captain C. R. Stevens, F.R.C.S., I.M.S., who was in charge of the cases, during my absence on other duty.

CONCLUSIONS.

1. The identity of what in India is called Cerebrospinal Fever with epidemic cerebrospinal meningitis is established, not only by the finding of the *Diplococcus intracellularis* in the Indian cases, but by the clinical and epidemiological aspects of the outbreaks, which exactly resemble outbreaks recorded in other countries.

2. A strong case appears to have been made out, in the present instance in the evidence recorded above, for connecting this disease with dust, either windborne, accumulated in roofs, etc., or produced in various processes of grain-cleaning.

3. In the Bhagalpur outbreaks no evidence was obtained pointing to direct contagion. In no instance were any of those in attendance on the sick attacked.

4. The disease was not associated with any prevalence of either pneumonia or influenza.

5. In no case could the disease be traced to any definite infection outside the Jail; the first cases in all three outbreaks were in persons who had been from six weeks to one year in jail.

6. Though a few wards produced a larger number of cases than others, there was no direct connection between the cases in these wards, nor between them and others, and often cases from the same ward came at long and irregular intervals.

7. The fact that 44 out of 47 cases came from forms of labour where there was great exposure to dust, and only 3 cases from the majority of the prisoners who were not so exposed, is difficult

of explanation unless we believe that dust either renders men more susceptible, or as is more probable, is the actual vehicle of the specific germ of the disease.

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AN OUTBREAK OF DIPHTHERIA CHECKED BY PROPHYLACTIC USE OF ANTITOXIN, AND THE ISOLATION OF INFECTED PERSONS.

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TOWARDS the end of October 1900 the Sanitary Authorities of Cambridge and Chesterton found themselves face to face with a serious outbreak of diphtheria; the first official notification had been on October 14th, and by October 23rd eleven cases had been notified, of which four terminated fatally, on October 15th, 21st, 22nd and 26th respectively. These four were all children attending the Infants' Department of a certain Higher Grade School, and the other seven cases were either children of this school or persons closely associated with them.

This limitation at the outset was very favourable to the taking of energetic measures, while the severe character of the outbreak made such measures imperative. The Medical Officer of Health, the Public Health Committee of the Borough Council and at their request the Pathological Department of the University, all combined to arrest the epidemic, and thanks to the hearty co-operation of the medical men of the town, their efforts were rewarded with a great measure of success.

On October 23rd preparations were made at the Pathological Laboratory for the bacteriological examination of suspected individuals and of those who had come in contact with them. A small body of students were got together to visit the school-children in their homes, to take swabs from their throats and from those of others living with them, and to recommend and administer a prophylactic injection of antitoxin whenever it seemed desirable. To R. H. Mayo, M.B., E. Ward, and P. R. Roy the thanks of all concerned are due for carrying out this part of the work.

On October 24th the children (about 60 in number) of the Infants' Department of the above-mentioned school, which had been closed two days previously, were visited in their homes, swabs were taken from their throats and from those of their brothers and sisters, and in many cases a prophylactic injection of antitoxin was given; several more of these children were injected on the following day, the result of the bacteriological examination of the swabs taken from them having been the detection of diphtheria bacilli, though there were as yet very trifling, if any, clinical symptoms of the disease.

A circular was then sent out to all the practising members of the Medical Profession in the town, informing them that the Public Health Committee had arranged for a free supply of antitoxin for both actual cases and "contacts," and that swabs might be obtained from the Pathological Laboratory, and when used sent there for bacteriological examination. Further they were requested to obtain three consecutive negative examinations from their convalescent patients before pronouncing them free from infection.

A few cases having occurred in two other schools, as many as possible of the children attending these schools were also visited in their homes and examined for diphtheria bacilli; and when later the epidemic was well in hand, in order to obtain a "control," swabs were obtained of 43 children attending a fourth school in which no case of diphtheria had occurred. The results of these examinations are reported in the next paper.

In all, over 950 bacteriological examinations were made of 650 persons; and 102 pure cultures were isolated, and tested for power of forming acid out of glucose and for virulence.

These measures were attended by a considerable amount of success; for though a large number of notifications continued to be made, many of them were founded on doubtful clinical signs together with the discovery of suspicious micro-organisms which ultimately proved to be pseudo-diphtheria bacilli. Only one death occurred among the cases notified after October 23rd.

The progress of the outbreak is shown in the following table of weekly notifications and deaths in Cambridge and Chesterton¹:

¹ The number of notifications of diphtheria in previous years may be of interest for comparison. From 1890 to 1899 inclusive they were as follows: 23, 20, 8, 15, 7, 24, 10, 16, 34, 27. In 1900 previous to the outbreak there had been 15 cases notified in the early part of the year, none in June, July or August, and one in September, which could not be connected in any way with the outbreak which followed. These figures are not *strictly*

TABLE I.

Week ending	Oct. 20	Cases notified	
		3	2 fatal
"	"	21	2 "
"	Nov. 3	26	1 "
"	"	8	0 "
"	"	2	0 "
"	"	0	0 "
"	Dec. 1	1	0 "
"	"	0	0 "
"	"	0	0 "
"	"	2	0 "
"	"	3	0 "
"	Jan. 5	1	0 "
Total		67	5

Mortality 7·5 per cent.

As soon as the disease had spread beyond the school in which the first cases occurred, the Public Health Committee arranged for the opening of an Isolation Home for the reception of children who were found to be harbouring the diphtheria bacillus without being themselves ill.

It was gratifying to find that the above-mentioned precautionary measures could be carried out without much difficulty. The parents with scarcely an exception allowed bacteriological examinations of their children to be made, and the great majority accepted injections of antitoxin when recommended, and after its nature had been explained to them. Over 100 injections were made by the laboratory staff alone, and no complaints of rashes or other unpleasant consequences reached the Sanitary Authority. Moreover, it was found possible in most cases to persuade parents to allow of the removal of their children to the Isolation Home.

The three consecutive negative examinations were not always obtained in the case of patients treated in their homes. Examples mentioned in the second paper show how necessary it is not to rely

comparable with those of the outbreak itself, because during the period of the latter bacteriological examination was for the first time extensively used, with the result that some mild cases, which would probably have otherwise escaped detection, were notified, while two notifications were withdrawn on the result of a negative bacteriological examination. The figures for the outbreak include all notifications, except those withdrawn, whether confirmed bacteriologically or not. For their analysis see the next paper. For the early part of this year since Jan. 5th the weekly notifications have been: 1, 1, 0, 2, 0, 1, 4, 1, 3.

upon a single negative examination, more particularly at a time when antiseptics are being applied to the pharynx. The great majority of patients were found to rapidly become free from the diphtheria bacillus during convalescence; but in two instances the bacilli have persisted for more than two months. It is exceedingly difficult to persuade such persons or their parents and guardians of the continued risk of infection. And the absence of symptoms or of any pharyngeal lesion is urged against it both by them and by some medical practitioners. It is nevertheless most important that such persons should continue to be isolated, more particularly when the bacilli which they harbour have been shown to be fully virulent. Many different kinds of antiseptics have been tried, but as yet no effective means of getting rid of the bacilli has been found.

The Origin of the Outbreak.

The distribution of infection in the first-mentioned school was traced to a case of chronic membranous rhinitis (G. N.). The origin of this case however could not be determined. About the time it commenced another of the school children (G. D.) fell ill with scarlet fever, and two months later while still in the Hospital for Infectious Diseases, where there were no known cases of diphtheria, the diphtheria bacillus was found in his throat. This then may have been a case of scarlet fever associated with diphtheria from its commencement, and if so, either boy may have been infected from the other, or both may have become infected from a common source.

While the actual origin of the outbreak has not been cleared up, there is no doubt about the way in which the infection became distributed: On October 23rd G. N. a member of the 3rd class of the Infant Department of the school already mentioned, was visited among other infants attending this school. He was found to be having tea with his brothers and sisters, and was in very good spirits, though he looked rather pale, and appeared to be suffering from nasal catarrh. His mother said he had had a "stuffy cold in the head" for about three weeks. During this time he had been regularly attending the school. Bacteriological examination revealed virulent diphtheria bacilli in great numbers in the nasal discharge, and after his removal to hospital, membrane was seen in the nose. His father, mother, sister, and brother, all the members of this family were found to have diphtheria bacilli in their throats. From three

of them (including G. N.) cultures were isolated and proved to be virulent. No less than seven of the nine male members of his school class (including himself and G. D.) suffered from diphtheria before October 23rd. The remaining two boys having been injected with antitoxin may have been saved by this means; though it is only fair to say that the pseudo-diphtheria bacillus was the only suspicious-looking micro-organism found in their throats.

It was notable that while seven of the nine boys of this class suffered from diphtheria, only one of the seven girls in it was affected, and one other was discovered to be harbouring a virulent diphtheria bacillus. For almost all their lessons, the boys and girls of the class were mixed indiscriminately, and according to their schoolmistress they played together out of school hours. The only explanation of the unequal incidence of the disease upon boys and girls was that twice a week the girls were separated from the boys to do needlework while the latter had a drawing lesson. It can scarcely be doubted that it was during this drawing lesson, when slates were in use, that the infection was distributed.

The following table shows the distribution of infected persons among the three classes of the "infants" attending this school:

TABLE II.

	Class I.		Class II.		Class III.	
	Boys	Girls	Boys	Girls	Boys	Girls
Cases	2	2	1	1	7	1
Deaths	1	0	1	0	2	0
Healthy children with diphtheria bacilli }	1	1	3	0	0	1
Healthy children without diphtheria bacilli }	15	13	7	4	2	5
Total No. of children in class }	18	16	11	5	9	7

The conclusions arrived at are incorporated with those at the end of the following paper.

Note on Chronic Membranous Rhinitis.

The name chronic membranous or fibrinous rhinitis has been applied to a certain class of cases of diphtheria in which the disease principally or solely affects the nasal mucous membrane, and is attended by little or no constitutional disturbance. Cases have been described on the Continent of Europe by Isambert, Concetti, Baginsky, and others, and in America by Park, Abbott, Ravenel, and Townsend.

References to the literature of the subject may be found in Ravenel's paper¹. Ravenel collected about 77 cases, in 41 of which there was a clear record of bacteriological examination, and in 33 the Klebs-Löffler bacillus was found. In all the cases the disease ran a benign course, and in all but a few the membrane was limited to the nose, constitutional symptoms being either slight or entirely absent.

Virulent diphtheria has but seldom been observed to have been contracted from contact with a case of membranous rhinitis. Park² mentions an instance of a child with only a slight nasal discharge in which diphtheria bacilli were present, giving rise to diphtheria in four children, two of whom died. The child with nasal discharge was a member of a family in which there had been a case of diphtheria three weeks before. Ravenel also gives the history of an instance of this kind.

On the other hand a case of membranous rhinitis has not unfrequently been observed to give rise to another of the same kind. Concetti³ obtained in two cases a history of direct infection from one to the other. Abbott⁴ found two children affected in the same family. Ravenel did the same, and gives a further instance in which two children and their mother were all affected.

That membranous rhinitis not unfrequently gives rise to the same condition, and has not more often been observed to cause severe diphtheria, may be explained on the assumption of a high degree of individual resistance in the several members of one family; for it is obvious that when a case has occurred it is the members of the same family who are most likely to be exposed to infection. On the other hand it may be that the diphtheria bacillus concerned in these cases has a lower degree of virulence than usual. This indeed has been found to be the case on several occasions. Thus Park tested the virulence of five cultures which were described by Abbott as all of a low degree of virulence⁵. Virulent diphtheria bacilli were found in two of Abbott's three cases. From the second case a culture which was not only devoid of virulence but was also of low vitality was obtained thirty days later. And from his third case, at a time when membrane was still present in the nose, a diphtheria bacillus was obtained which did not cause death, but produced only a local swelling, and a temporary indisposition from which the animals recovered. Ravenel tested cultures isolated from eight of his cases. Five were of the usual degree of virulence, one caused a slough as large as half-a-dollar from which the animal recovered, one caused a slough and death in 17 days, and one caused death in 15 days, but the lesions were not characteristic. A loopful of a serum culture was the dose used, the guinea-pigs weighing 350 g. and sometimes more. From the last case but one a virulent diphtheria bacillus had previously been isolated by Dr Kneass. In all of Townsend's four cases of pure membranous

¹ Mazýck P. Ravenel, "A Contribution to the Study of Membranous Rhinitis." *The (Philadelphia) Medical News*, 25 May, 1895.

² Cited by W. H. Welch, "Bacteriological Investigations of Diphtheria in the United States." *Am. Journ. of Med. Sciences*, Oct. 1894.

³ Cited by Abbott.

⁴ A. C. Abbott, "The Etiology of Membranous Rhinitis." *The (Philadelphia) Medical News*, May 1893.

⁵ One killed a guinea-pig in 4 days, two, each in 5 days, and two caused symptoms from which the animals recovered. Cited by Welch (*loc. cit.*), the dose not being stated.

rhinitis the diphtheria bacilli had the usual degree of virulence¹. To these may be added some cases cited by Abbott. Concetti tested the virulence of cultures obtained from two cases, Stamm that of cultures from four cases, and Baginsky that of cultures from two cases. All these are described as virulent. From the case which occurred in Cambridge a highly virulent culture was obtained (see next paper, Table III. G. N. i.). Thus from 29 cases of membranous rhinitis mentioned here 21 have yielded virulent diphtheria bacilli, and 8 bacilli more or less attenuated. From two of the 21 cases moreover attenuated cultures were obtained at a later period.

	Virulent	Attenuated
Park	0	5
Abbott	2	1
Ravenel	6	2
Townsend	4	0
Concetti	2	0
Stamm	4	0
Baginsky	2	0
Cobbett	1	0

¹ Cited by Welch.

THE RESULT OF 950 BACTERIOLOGICAL EXAMINATIONS
FOR DIPHTHERIA BACILLI DURING AN OUTBREAK
OF DIPHTHERIA AT CAMBRIDGE AND CHESTERTON.

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THE following is an account of the bacteriological examinations for diphtheria bacilli made by me at the University Pathological Laboratory, from October 23, 1900 to Jan. 5, 1901; a period which includes all but the first week of the outbreak described in the preceding paper.

The number of persons examined was 692. Some of these having been tested several times, the total number of examinations made exceeded 950.

Among the 692 persons examined, there were 42 notified cases of diphtheria¹. Besides these there were about 22 other notified cases in the town which were either sent direct to the hospital without being examined by me, or had been taken ill before I began my work.

Bacteriological examinations for diphtheria bacilli in the throats and noses of suspected cases of diphtheria, and those who have been exposed to infection require to be at once rapid and accurate. Valuable as these examinations undoubtedly are, it must be admitted that they are attended with some difficulty, and in unskilled hands have given rise in the past to not a little inconvenience and pecuniary loss. They have consequently become looked upon with some distrust, not only by a portion of the general public, but also by not a few medical practitioners. The frequent occurrence of the pseudo-diphtheria bacillus of

¹ Including one from a neighbouring village, and excluding four cases in which symptoms of diphtheria were absent or uncertain and which were notified because suspicious bacilli—which were afterwards recognised as Hofmann's bacilli—had been found.

Hofmann¹ in cultures from the human pharynx and nose is the chief source of difficulty². It is true that there are two reliable tests which serve to distinguish this bacillus from the bacillus of diphtheria, namely the absence of acid-formation in glucose media, and the absence of pathogenic power, particularly the power to produce inflammatory oedema at the seat of subcutaneous inoculation in guinea-pigs. But each requires time, and the opinion of the bacteriologist to be of practical value often cannot await the preparation of pure cultures and the injection of animals,—to say nothing of the expense of such investigations when carried out on the large scale which is often desirable. What then is to be done? The answer I believe to be that it is possible to train the eye to distinguish, with a sufficient degree of precision, the diphtheria bacillus from the bacillus of Hofmann. But I think that the eye cannot become sufficiently trained for this purpose unless the observer frequently tests the opinions which he forms on morphological grounds, by isolating his cultures and testing them in various ways, including the injection of animals. Such tests should never be omitted in cases of doubt or when much depends upon the issue. Further the making of drawings with the aid of the camera lucida is of the greatest assistance in training the eye, because it concentrates the attention upon individual bacilli for a far longer time than would otherwise be the case, and because it gives the observer the opportunity of comparing his

¹ The pseudo-diphtheria bacillus first described by Löffler, and afterwards by Hofmann and others, is called throughout this paper by the name of Hofmann, in order to clearly distinguish it from the true diphtheria bacillus with which the name of Löffler is usually associated.

² Roux and Yersin (*Ann. de l'Inst. Pasteur*, 1890, vol. iv. p. 385), ten years ago expressed the opinion that the frequent presence of the pseudo-diphtheria bacillus in the mouths of healthy persons and others not suffering from diphtheria, does not interfere with the bacteriological search for the true bacillus. They arrived at this opinion because of the small number of colonies of the "pseudo-" bacillus when present. It is doubtless true as a rule that the colonies of this micro-organism are not numerous. But I have several times found cultures from the nose and pharynx consisting of very numerous colonies almost all of which were made by the bacillus of Hofmann, and on the other hand from cases of diphtheria both active and convalescent I have had cultures with only one or two colonies of diphtheria bacilli. Roux and Yersin stated that their pseudo-bacilli produced acid, but their description would seem to include the Hofmann bacillus. They wrote before the importance of the acid test had been recognised, and it is probable from the frequency with which they found their pseudo-diphtheria bacilli (in 40 per cent. of the children of a school in a healthy country village) that their group of pseudo-diphtheria bacilli included both the attenuated diphtheria bacillus and the bacillus of Hofmann.

observations with one another. All these means have been used in the investigation with which this paper deals.

The chief interest of the investigation centres in the large number of children of school age examined, coming from four schools, which in varying degrees had been invaded by the disease. These may be arranged as follows

	Number examined.
(1) Park Street School (14 cases of diphtheria with 4 deaths) ...	51
Young brothers and sisters of the Park Street School scholars (5 cases)	46
(2) King Street School (2 cases)	59
Young brothers and sisters of the above	33
(3) Occupation Road School (2 cases)	57
Young brothers and sisters of the above	32
(4) Romsey Town School and the young brothers and sisters of the scholars (0 cases). No cases of diphtheria occurred in the part of the town in which this school is situated	43
	321

Methods.

The nutrient medium used was alkalisied serum to which 1% of glucose had been added¹. Horse-serum was used by preference, but owing to the large demand a good deal of ox-serum had to be used as well. Both media proved convenient and reliable. The earliest appearance of a visible growth of diphtheria bacilli was observed in six hours, and the latest in three days. In the latter case the delay was thought to have been caused by the application of a weak carbolic acid solution to the throat previous to the application of the swab². The cultures were examined the day after they were sown and if no growth of any kind had appeared they were re-examined on several days next following.

For staining Löffler's methylene-blue was used diluted with 2 or 3 volumes of water, the cover-glass preparations being mounted in the staining-fluid. The groups of bacilli were observed to decolorise the fluid around them, and they consequently appeared as if mounted in a colourless fluid. This method was selected on account of its quickness, and because specimens so mounted seemed to me to present a more characteristic appearance than if they are washed and dried after staining and mounted in Canada balsam. It was found practical to make 4 to 9 separate preparations on one cover-glass from the different colonies of a single culture-tube. The examinations thereby gained in thoroughness and took less time.

¹ First suggested by Prof. Lorrain Smith of Belfast. For the preparation of this nutrient medium see *Brit. Med. Journ.* 1894, vol. ii. p. 1177.

² Experience gained during this investigation confirmed the generally accepted opinion that antiseptics applied to the throat not unfrequently render the bacteriological examination useless or misleading. Practitioners can scarcely be reminded too often of this fact.

Cover-glass preparations from *broth* cultures were, before being stained, dipped in 5% solution of acetic acid for ten seconds, and washed in water to which a few drops of ammonia had been added to more rapidly remove the acid.

When bacilli more or less resembling the diphtheria bacillus were found, the fact was notified to the physician, if any was in attendance, and the microscopic preparation preserved for future reference. Steps were taken to obtain a pure culture for further investigation, but owing to the pressure of work during the early days of the investigation when the number of tubes to be examined daily was very great (on one occasion exceeding 100), the attempt to get a pure culture was in some instances omitted or failed. Nevertheless of 250 cultures of more or less suspected bacilli, over 100 were isolated and tested on animals.

The suspicious bacilli found were provisionally classified on the result of a microscopic examination into: (A) long bacilli, microscopically identical with the diphtheria bacillus, and (B) and (C) shorter bacilli which differed from, but had more or less resemblance to the diphtheria bacillus.

When pure cultures had been obtained the bacilli were tested for their power to produce an acid reaction to litmus, when grown for 48 hours in broth containing 1% of glucose and having an initial alkalinity to litmus equal to about 7 c.c. of normal alkali per litre.

This was followed by injection of broth cultures into guinea-pigs.

The quantity injected was regulated by the reaction shown by the glucose-broth culture; thus, if this had been acid, 0.1 c.c. was the quantity usually employed, if alkaline 2.0 c.c. In a few instances the smallest dose injected of a culture which had shown itself capable of producing acid was 0.5 c.c. The number of injections in the case of each culture, the dose and age of the culture, and the size of the guinea-pigs used are shown in Table III. In all cases the cultures injected were grown in sugar-free broth.

Autopsies on the animals were made in nearly all cases, and since the subcutaneous oedema at the seat of inoculation, the excess of fluid in the pleural cavities, and the reddening of the suprarenals, are so characteristic of death from diphtheria, it was not thought necessary as a rule to practise the injection of culture plus antitoxin.

A description was kept of the naked eye and microscopical appearances of all cultures on serum and in glucose and sugar-free broth. The microscopic preparations of all were kept, and in some instances the serum cultures were sealed with the blowpipe and preserved for reference. When the stress of work became gradually relaxed, drawings were made with the aid of a camera lucida of bacilli from serum, glucose broth, and the sugar-free broth culture injected into the guinea-pigs. And this was done as a routine measure during the latter half of the investigation.

The Morphology of the Diphtheria and Pseudo-diphtheria Bacilli.

The range of variation in form of the diphtheria bacillus is unfortunately great. Wesbrook, Wilson and McDaniel¹ have recently drawn attention to this, and have published drawings of 19 different shapes which this micro-organism may assume. Briefly they recognise three main types, which they call (1) granular, (2) barred and (3) solid colour forms. The last-mentioned contain ("Type D²") bacilli which generally present the appearance of pairs with opposing extremities flattened and thickened, the distal extremities bluntly pointed or abruptly rounded. Whether the colourless part between the two opposed members of the pair is an actual space between two bacilli or an unstained part of one bacillus they are unable to state. They consider that "these bacilli are probably included under the pseudo-diphtheria or Hofmann group of other observers." Yet in spite of their innocent appearance they were the most prevalent form in an outbreak of diphtheria at Owatonna examined by Wesbrook, and are occasionally the only form present in clinical cases, and frequently pathogenic to guinea-pigs. Prof. Sims Woodhead tells me that he has met with very virulent bacilli of similar appearance. During the recent outbreak here they were never observed.

If I may be permitted to go once again over familiar ground I shall venture to describe briefly the various types of diphtheria and pseudo-diphtheria bacilli, as they have appeared to my eye during my recent observations.

To begin with the pseudo-diphtheria bacillus. A micro-organism which I take to be the pseudo-diphtheria bacillus of Löffler and Hofmann, very common in the pharynx and nasal cavities among the poorer classes in Cambridge, which forms no acid in glucose culture media, and causes no oedema in the guinea-pig, has the following appearance under the microscope. From young serum cultures it appears with considerable regularity as a darkly staining oval bacillus of somewhat variable length, with one (or rarely more) narrow unstained septum. These bacilli present a very characteristic appearance and do not at all closely resemble the common adult forms of the true diphtheria bacillus. Occasionally, however, colonies are met with which contain a fair number of bacilli with several septa, and the differential diagnosis is then more difficult (see Pl. V., Figs. 8,

¹ *Trans. of the Assoc. of American Physicians*, 1900.

11 and 12). In broth cultures I think this bacillus more closely resembles the diphtheria bacillus. Here too the oval bacillus with one unstained septum usually predominates, but giant forms which closely resemble the diphtheria bacillus are generally present also and may be numerous. They are many times the length of the oval bacillus, often clubbed, and are, I think, rather thicker than the diphtheria bacillus (see Pl. V., Fig. 4). It is cultures in which these giant forms predominate which present the closest resemblance to diphtheria bacilli.

The true diphtheria bacilli in young serum cultures vary greatly. Short forms like those just described were frequently seen, but I have not yet found them the only forms present in a virulent culture. I regard them as young forms. Like Westbrook I found that the commonest type of diphtheria bacillus in original serum cultures was the granular or, as I prefer to call it, the *beaded bacillus*. This is a faintly staining curved bacillus of irregular width, usually two or three times the length of Hofmann's bacillus, with one or more darkly staining rounded dots, often terminal, sometimes central (see Pl. III., Figs. 6 and 10, and Pl. IV., Fig. 7). Next in frequency was the *barred or segmented bacillus*. It varies in length but is always longer than Hofmann's bacillus, is curved and of irregular thickness. It stains darkly with several narrow unstained or faintly stained linear intervals. One of the roughly rectangular segments is often larger than the others (see Pl. III., Fig. 5). If the enlarged segment is terminal the bacillus is club-shaped, if mesial spindle-shaped.

Intermediate between these types were the "streptococcus forms" (Pl. III., Fig. 3) which may appear as a row of dots at regular intervals in a pale bacillus. If faintly stained the dots alone may be seen and the bacilli may indeed be taken for streptococci; but the regular and somewhat stiff curve which they assume, and the uniformity of their length and finally their 'arrangement' will suggest their true nature, and in case of doubt re-staining more darkly will remove all difficulty.

Some cultures were formed of bacilli so uniformly stained that it was difficult to see any trace of segmentation and granulation (Pl. III., Fig. 1). Thus it will be seen that I learned to recognise five types of diphtheria bacilli from young serum cultures,

- (1) Oval bacilli with one unstained septum. Young forms.
- (2) Long, faintly stained, irregularly beaded bacilli.
- (3) Regularly beaded bacilli. Streptococcal forms.
- (4) Segmented bacilli.
- (5) Uniformly stained bacilli.

In general the characteristics of the bacillus other than beading and segmentation were that they were more or less curved and of varying thickness. The "arrangement" varies; occasionally the bacilli resembled a lot of pine needles on the ground. Broth cultures were apt to be much clumped. In any one culture the bacilli, if we exclude what I take to be young forms, conformed fairly closely to one type, and one could often recognise the same type in successive cultures from the same person. On glucose broth, when it had become acid, extraordinary forms sometimes appeared with one enormously swollen segment either at the end or in the middle. These very long forms with a swollen extremity resembled serpents, while the shorter forms with a more central swelling were something like elongated peg-tops (see Pl. IV., Figs. 4 and 9).

When once one had become well acquainted with its range of variation it was fairly easy to recognise the diphtheria bacillus and to distinguish it from all others (that is, if the acid-producing but non-virulent bacillus which resembles it in all other ways be admitted as an attenuated diphtheria bacillus). The bacillus of Hofmann was the only one which presented any difficulty, and about this I slowly became persuaded that it could as a rule be excluded on morphological grounds alone. This of course would not have been the case had I met with the short Hofmann-like yet virulent diphtheria bacillus described by Westbrook, but it did not occur, and it is worthy of note that 69 cultures classed provisionally under B or C (bacilli morphologically resembling Hofmann's bacillus) were isolated and tested, with the result that not one produced any acid in glucose broth or caused oedema in the guinea-pig¹. This result was somewhat unexpected, for I had anticipated that among them would have been found some true diphtheria bacilli. But this was not so, and the investigation of the pure cultures which were isolated showed that all the true diphtheria bacilli among them, together possibly with four Hofmanns², had been

¹ It is true that 8 out of 83 animals injected died within 10 days of inoculation, but that they did not die from the bacillus in question is clearly shown by the following considerations, (a) there was no oedema at the seat of inoculation in any during life, (b) nor after death, and there was no excess of pleural fluid, (c) the injections were in all cases repeated, sometimes in larger doses, and the animals remained well, (d) though the 83 injections occupied two months and more, all deaths happened during one week, and were probably therefore due to some cause other than the injection.

² In these four instances it seemed probable that the bacilli first seen and classed under A were true diphtheria bacilli, and that the attempt to isolate them failed and resulted in isolating a pseudo-diphtheria bacillus instead.

classified on microscopical grounds under A, and that classes B and C contained none but the non-acid-forming pseudo-diphtheria bacillus.

These harmless bacilli, which I take to be the pseudo-diphtheria bacilli of Löffler and Hofmann, were as I have said easily distinguished from the true diphtheria bacilli found during this epidemic when grown on alkalisied serum. Their colonies on serum usually became after a few days larger and whiter than those of the diphtheria bacillus but there was often little or no difference at the end of 24 hours, and in other respects in their mode of growth in broth and on gelatine they closely resembled the true diphtheria bacillus, except that they grew more quickly. They did not produce a definite film on broth, but it is rare for diphtheria bacilli to do so until they have become well accustomed to the medium.

*The Distribution of the Diphtheria and Pseudo-Diphtheria
Bacilli among those examined.*

692 persons were examined, including 42 notified cases of diphtheria. Among these 42 notified cases no suspicious micro-organisms were found in 6. From 5 Hofmann's bacillus unassociated with the diphtheria bacillus was isolated and proved to be incapable of producing acid, or causing oedema in guinea-pigs. The diphtheria bacillus was found in 24 (57%)¹. From 16 of these 24 cases 20 pure cultures were isolated, and all but one proved to be highly virulent. In this latter instance the bacillus which was morphologically identical with the virulent diphtheria bacillus, and formed acid in glucose broth, was obtained from a patient examined for the first time during convalescence from diphtheria. From the remaining 7 cases bacilli more or less closely resembling the short diphtheria bacillus were found, and classified provisionally under B. These were not tested on animals but from subsequent experiments with similar cultures I should say that the majority were probably Hofmann's bacilli.

Among the 650 other persons, bacilli morphologically identical with the diphtheria bacillus were found in 19. From 8 of these 19 persons 9 pure cultures were obtained. All produced acid and all but three

¹ Among over 5000 suspected cases of diphtheria in New York Park and Beebe found the diphtheria bacillus in about 60%. Morse found it in 72% of 301 cases of diphtheria in the Boston City hospital. (Cited by Welch, "Bacteriological Investigation of Diphtheria in the United States," *Am. Journ. of Med. Sci.* Oct. 1894.)

were highly virulent. These latter were entirely without virulence. In addition to these, four attempts, previously mentioned, to isolate a diphtheria bacillus which had been recognised as such on the strength of a microscopic examination of the original culture, failed, and only the bacillus of Hofmann was obtained (proved in all these cases to be non-virulent). It may be that Hofmann's bacillus was mistaken for the diphtheria bacillus at the first examination, but I think it more probable that both the true and the pseudo-diphtheria bacillus were originally present and attempts to obtain a pure culture of the former resulted in isolating the latter only.

Of these 19 persons, who were not notified cases of diphtheria, and in whose throats diphtheria bacilli (proved to be virulent in five cases) were found, a few had slight sore throat at the time of examination, and all but one received injections of antitoxin. Without this some would doubtless have developed into clinical cases of diphtheria. It is therefore impossible to say how often diphtheria bacilli were found in healthy persons. But this much may be stated that *diphtheria bacilli were found only in actual cases of diphtheria, or among those who had come directly into contact with such cases.* These latter were either children attending the school most affected, or inmates of houses where there was an actual case. Among a very large number of other people examined the diphtheria bacillus was found not once.

The Hofmann's bacillus was found altogether 157 times, and isolated in pure culture 69 times. All these 69 cultures failed to produce acid in glucose broth, and caused no local oedema in guinea-pigs. It was certainly not more frequently found among those who had come into contact with diphtheria, than among those who had not. It was less frequently found among the scholars of the "higher grade" school where there was much diphtheria, than among the scholars of ordinary schools where there was little or none¹. Among the small number of children of the upper classes examined it was conspicuous by its rarity.

The 25 cultures isolated which proved to be virulent, killed guinea-pigs of 200—500 g. in two or three days, when 0·1 c.c. of a 48 hours' broth culture was injected. (A few of the earlier experiments were made with older cultures and in four instances the smallest dose given was 0·5 c.c.) In three instances 0·1 c.c. of a 48 hours' culture failed to kill, but these cultures were very poorly grown, and when the

¹ Roux and Yersin have made a similar observation.

injection was repeated a few days later, after the culture had passed through one or more generations in broth, and had become more accustomed to this medium, death took place within forty-eight hours. It may therefore be stated that between the cultures which killed in doses of 0.1 c.c. and those which were entirely harmless in doses of 2.0 c.c. there were none with intermediate degrees of virulence. The ratio of non-virulent diphtheria bacilli to virulent diphtheria bacilli found was 4 : 25, or 16 %. Even if these non-virulent diphtheria bacilli be entirely harmless to man and incapable of becoming virulent, this relative frequency of occurrence would not seriously impair the value of the bacteriological test. The case of Hofmann's bacillus is quite different unless it be excluded, for it occurred six times as often as the diphtheria bacillus, or in about 23 per cent. of the healthy people examined.

From the above account it will be seen that the bacilli isolated and tested may be classified as follows¹.

1. Bacilli, identical in appearance both in culture and under the microscope with the diphtheria bacillus.

(a) Pathogenic acid-producers = virulent Klebs-Löffler bacilli 25

(b) Non-pathogenic acid-producers = the so-called attenuated diphtheria bacilli 4

2. Bacilli somewhat resembling, but shorter and stouter than the diphtheria bacilli.

Non-pathogenic, non-acid-producers, Löffler-Hofmann, so-called Pseudo-diphtheria bacilli 69

This classification was first made by Park and Beebe².

Is the Pseudo-Diphtheria Bacillus of Löffler and Hofmann an attenuated Diphtheria Bacillus capable of becoming dangerous?

This is a most important question, for on its answer depends the whole basis of the measures which should be taken for combating diphtheria. For if the so frequent Hofmann bacillus can under certain conditions, say insanitary environment, become the true diphtheria bacillus, then diphtheria must be combated by general sanitary measures, and it is

¹ During the past six years I have met with other bacilli which more or less resemble the diphtheria bacillus. These mostly came from the skin. The above-mentioned types are the only diphtheroid forms which I have found in the nose or pharynx.

² *New York Med. Rec.* XLVI. 1894, p. 385.

impossible to seek out and strictly isolate those who have these micro-organisms in their mouths. On the other hand, if the Hofmann bacillus is entirely harmless, then isolation of those who carry about dangerous bacilli is possible and reasonable and should be strictly enforced, even at the cost of some individual hardship.

Roux and Yersin, Hewlett and Knight¹, Richmond and Salter² and others have laid stress on the frequency with which the pseudo-diphtheria bacillus is found in the mouths of persons convalescing from diphtheria, and its relative infrequency during the acute stage of the disease. But surely it is often overlooked in the early stage of the attack because one then discovers the virulent bacillus so easily and does not trouble to look any more. On the other hand when the diphtheria bacilli are disappearing and are hard to find, a long and careful search is made, and the pseudo-diphtheria bacillus previously overlooked is seen for the first time. I have frequently tested on animals these bacilli occurring during convalescence and have always found them to be as completely devoid of virulence as those derived from healthy persons. Roux and Yersin speak of diphtheria bacilli intermediate in virulence between the pseudo and the fully virulent diphtheria bacillus. Such may well exist if the acid-forming non-virulent bacillus is an attenuated diphtheria bacillus. These bacilli of low virulence have not been met with in Cambridge, and the non-virulent diphtheria bacillus was rare. But the bacilli which we are now considering are those which do not produce acid out of glucose. These are in my experience always devoid of virulence, and have never been found to cause local oedema even in relatively large doses (2·0 c.c. of cultures rich in bacilli).

The crucial question is, Can the diphtheria bacillus be converted into the bacillus of Hofmann, and can the bacillus of Hofmann become the virulent diphtheria bacillus? Neither question can be definitely answered. Roux and Yersin starting with virulent cultures of diphtheria bacilli, and growing them under unfavourable conditions of temperature in a current of air, obtained a non-pathogenic bacillus which produced no toxin. Sometimes the change was quick, sometimes slow, and it is significant that they did not produce intermediate degrees of virulence as regularly as in the attenuation of the bacillus of anthrax. In one instance, however, the virulent bacillus became replaced by one which produced marked oedema but did not kill,

¹ *Trans. Brit. Inst. Prev. Med.* first series, p. 7, 1897.

² *Guy's Hospital Reports*, vol. LIII. p. 55, 1898.

before its virulence finally disappeared. But this attenuation proves nothing but that the diphtheria bacillus may lose its virulence, which is not denied, but that it should turn into the *pseudo-diphtheria* bacillus is quite another matter. Hewlett and Knight believed that they were able to produce this transformation on one occasion, but attempts to repeat this experience with other bacilli were not so successful. In the one successful instance they reject but they do not exclude the possibility that they may have started with a mixture of the two bacilli.

Attempts to make the pseudo-diphtheria bacillus virulent have been recorded. Roux and Yersin could increase the virulence of a bacillus which caused oedema, though it did not kill, but were unable to give virulence to non-virulent forms. Hewlett and Knight believed that they were able in one or two cases to transform the pseudo-diphtheria bacillus of Hofmann into the virulent diphtheria bacillus. In the one experiment which they record the culture passed through a large number of generations in tubes and plates as well as in animals. For a long time it remained inoffensive, and when it became virulent it did so suddenly and for no better reason than that the 19th and 20th generations were serum cultures incubated at 37° for a week. This acquisition of virulence was associated with the appearance for the first time of the faculty of forming acid. In 1898 Richmond and Salter (*loc. cit.*) published a paper on "The Etiological Significance of the Diphtheria Bacillus and its Variants" in which they briefly stated that they had succeeded in transforming the pseudo-diphtheria bacillus of Löffler and Hofmann into the virulent diphtheria bacillus by repeated passage through certain birds. The transformation was gradual, the bacilli becoming longer, staining differentially with methylene-blue, and ultimately forming acid in neutral broth, and killing guinea-pigs with all the pathognomonic signs of experimental diphtheria. More recently Salter¹ has described in detail the conversion of one of the four Hofmanns which he transformed. The bacillus employed came from a case of post-scarlatinal diphtheria. Morphologically it was a typical Hofmann's bacillus. It produced an acid reaction in broth, and was entirely harmless to guinea-pigs. After its exaltation it was definitely but not strongly toxic for these animals, 5.0 c.c. of a broth culture producing death on the sixth day. The usual gelatinous oedema at the seat of inoculation, pleural exudation and

¹ *Trans. Jenner. Inst. Prev. Med.* Second Series, p. 113, 1899.

reddening of suprarenals was found after death. Its pathogenic action was counteracted by antitoxin.

Salter was moreover able to kill guinea-pigs with mixtures of diphtheria toxin and antitoxin which contained just sufficient of the latter to prevent death, by adding small quantities of filtered culture of (unconverted) Hofmann's bacilli. From this he concluded that diphtheria protoxoid is a common product both of the pseudo-diphtheria and of the diphtheria bacillus.

In view of the wide distribution of Hofmann's bacillus among healthy persons in Cambridge and elsewhere the conclusion arrived at by Richmond and Salter that the pseudo-diphtheria bacillus is an attenuated variety of the true causal agent of diphtheria is, if well founded, of great importance. But until the position of the bacillus of Hofmann has been clearly established, and it has been proved capable of being converted into the virulent diphtheria bacillus, not merely by laboratory procedures, but further, under natural conditions, we must not conclude that the causal agent of diphtheria is widespread, nor weaken in our efforts to find out those who harbour the *virulent* diphtheria bacilli, and to isolate them until they have become freed from these micro-organisms.

There is no evidence that bad drains and insanitary environment can ever convert non-virulent into virulent bacilli and originate diphtheria. Though the influence of climate and season is marked, it has not been shown that it is exerted upon the bacillus rather than upon the human being. All experience goes to show that except in occasional instances, such as where it is distributed by milk, or contracted from animals, cases of diphtheria are attributable to the bacilli being conveyed, in most instances directly, from the one person to another. The following is a good instance of what I believe is the common mode of transmission.

B. C. aged 14, was taken ill with headache and sore throat on December 26th. On the 27th a patch of membrane was seen on the left tonsil. An injection of antitoxin (2400 units in 3 c.c.) reduced his temperature from 101·5° to 97·6° F. within four hours, and removed all the constitutional symptoms. The latter did not return, but a condition of follicular tonsillitis associated with the continued presence of the diphtheria bacillus persisted for many days. A cultivation taken on the 27th gave the diphtheria bacillus. A similar culture was obtained from his sister who remained well.

The day before B. C. was taken ill he had spent the evening with some neighbours. Four of these were examined for diphtheria bacilli with the result that they

were found in two boys of 16 and 20, but not in a baby, nor yet in a sister of 14. On further enquiry it was discovered that B. C. had played with one of these boys at parlour cricket, and each had taken it in turn to score with the same pencil, which doubtless often found its way into their mouths. It is quite clear then how the bacilli found their way from B. C. to this boy. From him it easily found its way to his brother, for the two boys slept in the same bed.

The Need of more than one Negative Bacteriological Examination of Patients convalescent from Diphtheria.

The Boston Board of Health, U.S.A., require two consecutive negative examinations of convalescents before they are pronounced free from infection, and for hospital patients the rule is three consecutive negatives¹. During the recent epidemic here, the medical practitioners have been requested to submit swabs from their convalescent patients until three consecutive negative examinations have been obtained. Table II. shows a number of these consecutive examinations. It will be seen that more than once two consecutive negative examinations were followed by the finding of virulent diphtheria bacilli. It was thought that some of the misleading negative examinations were caused by the swabs being taken too soon after the application of some antiseptic. But it may be that in some cases the diphtheria bacillus lurks in the nasal cavities, or perhaps in one of the sinuses communicating with them, and only occasionally finds its way into the pharynx. The fact that after pharyngeal diphtheria the bacillus has been found in the nose when it was not detected in the pharynx seems to support this suggestion².

The need of more than one negative examination is clearly shown by the following history. *P* a scholar attending the school in which diphtheria had broken out and *Q* his baby brother were examined on October 24th, and diphtheria bacilli were found in the boy, but not in the baby. *P* who was staying away from home received a dose of antitoxin and remained well, but *Q* received none. A short time after *P* had returned home *Q* got diphtheria. When he had recovered, another

¹ See paper by H. W. Hill, M.D., *Journ. of the Massachusetts Association of Boards of Health*, vol. VIII., Oct. 1898.

² Wolff in 1895 examined bacteriologically the accessory sinuses of the nose in fatal cases of diphtheria etc. The *B. diphtheriæ* was found in 12 out of 22 cases of diphtheria in one or more of these sinuses, including once in the frontal, and six times in the sphenoidal sinus. Cited by Howard and Ingersoll. *Am. Journ. of Med. Sciences*. May, 1898.

examination of the two children was made on November 21st and the result was negative in each case. The physician in charge was satisfied with this single negative result and after this no precautions were taken to isolate the children any longer.

Three weeks to a month later two other boys, *X* and *I*, fell ill with diphtheria within a few days of each other. One of them (*X*) had been going to the house of *P* and *Q* to have music lessons from the father. It was *I*, however, who was taken ill first.

It was thought possible that the boy who had the music lessons had got the infection at the house of *P* and *Q*—though he had seen no one there but the father—and had passed it on to his brother, who being perhaps a little more susceptible was the first to fall ill. Consequently another series of examinations of the *P* and *Q* family was made on December 21st, with the result that diphtheria bacilli were found in *P* and *Q*, the mother, and the maidservant. The father and a young sister were the only members of the household free from infection. It is interesting to note that these two alone had never come into contact with the baby when he was ill.

The following table shews the consecutive examinations of the members of this household.

TABLE I.

D=A clinical case of diphtheria.

Δ=The diphtheria bacillus diagnosed on morphological grounds.

Δ= do. do. isolated and proved virulent.

H=The bacillus of Hofmann.

H= do. do. isolated and proved not virulent and incapable of producing acid.

O=No suspicious micro-organisms.

○=No growth of any kind.

Name and reference number		Consecutive examinations					
Father	786				27. XII ○	31. XII O	8. I H
Mother					21. XII Δ	2. I O	8. I Δ
P.	17	24. x Δ	7. xi Δ	21. xi ○	21. XII Δ	2. I H	8. I Δ
Q.	74 D	24. x O	10. xi O	21. xi O	21. XII Δ	2. I H	8. I H
Sister	73	24. x O			21. XII O	2. I H	8. I H
Maid	779				21. XII Δ	2. I O	8. I O

The history just related affords also a good instance of the occasional long persistence of the virulent diphtheria bacilli in the pharynx. During the outbreak at Cambridge the bacilli as a rule were found to disappear very quickly from the throats of convalescents. In one man

however they persisted from October 31 to January 2nd. No attenuation of the bacilli has been observed in this case, nor in that of the boy *P*. Non-virulent diphtheria bacilli, as has already been mentioned, were but four times observed, once only in a convalescent and three times in a contacts who remained well.

The following Table shows some of the results of consecutive examinations and will serve to illustrate the necessity of more than one negative examination, and the persistency of the diphtheria bacillus in a number of cases.

TABLE II.

RESULTS OF SOME CONSECUTIVE EXAMINATIONS OF DIPHTHERIA PATIENTS AND OTHERS WHO WERE FOUND TO HARBOUR THE DIPHTHERIA BACILLUS.

Initials and reference number	D = clinical case of diphtheria	Consecutive examinations										Remarks
		Δ = Diphtheria bacillus proved by injection into animals. H = Hofmann's bacillus proved by injection into animals. O = No suspicious bacilli.										
		Δ = Do. microscopic examination only. H = Do. microscopic examination only. O = No growth of any kind on the culture tube.										
G. N. 1	D	23. x Δ	7. xi O	13. xi O	15. xi Δ	20. xi O	24. xi O	27. xi O	19. xi O	Chron. membr. rhinitis		
Gl. N. 58		24. x O	31. x O	3. xi Δ	19. xi O	10. xi H	14. xi O	16. xi O				
T. N. 59		24. x O	31. x Δ	3. xi H	10. xi O	15. xi O	16. xi O	16. xi O				
Mr N. 302	D		31. x O	3. x Δ	10. xi O	15. xi O	16. xi O					
Mrs N. 57	D	25. x Δ	31. x O	3. xi O	10. xi O	15. xi O	16. xi O					
Ms A. 3	D	23. x Δ	6. xi O	7. xi O	16. xi O							
A. G. 3												
J. S. 9		24. x Δ	3. xi Δ	10. xi O	17. xi O	27. xi O						
Jk. S. 82		24. x O	3. xi H	10. xi H	17. xi H	27. xi O						
G. S. 83		24. x O	3. xi H	10. xi H	17. xi O	27. xi H						
D. S. 27		24. x O	3. xi H	10. xi H	17. xi O	27. xi H						
M. D. W. 45	D	24. x H	28. x H	7. xi O	12. xi O	14. xi H	16. xi O	19. xi O	22. xi O	24. xi O		
M. C. 47	D	24. x Δ	7. xi O	13. xi Δ	15. xi O	17. xi O	20. xi O	17. xi O	20. xi O	22. xi O	24. xi O	
H. C. 115	D	26. x O	29. x Δ	31. x O	2. xi O	13. xi O	15. xi O					
M. H. 124		27. x H	7. xi O	12. xi O	14. xi O	16. xi O	24. xi O			22. xi O 24. xi O		
A. B. 172	D	28. x H	29. x H	7. xi H	12. xi O	14. xi O	16. xi O	19. xi O	24. xi O	Δ ¹ = non-pathogenic acid producing bacillus		
A. M. 260		30. x Δ ¹	3. xi O	8. xi H	12. xi H	14. xi O	16. xi O	19. xi O	24. xi O	27. xi H		
S. M. 304		31. x H	8. xi H	12. xi H	14. xi O	16. xi O	19. xi H	22. xi O	24. xi O			
W. N. 584		5. xi H	12. xi H	14. xi O	16. xi O	19. xi H	22. xi O	24. xi O	27. xi O			
Q. D. 283		30. x Δ	7. xi H	12. xi O	14. xi O	16. xi O						
G. D. 244	D	29. x Δ	18. xi H	20. xi H	27. xi H	30. xi Δ	18. xii O			Case of "scarlet fever"		
Mrs M. 250		29. x Δ	12. xi Δ	24. xi O	2. i Δ							
Mr J. 299		31. x Δ	18. xi Δ	20. xii Δ	2. i Δ							
G. D. 714	nose pharynx	5. xii H	11. xii Δ	15. xii O	20. xii H	21. xii O						
		5. xii H	11. xii O	15. xii O	20. xii H	21. xii O						
M. Cl. 723	D	1. xii Δ	17. xii Δ	20. xii Δ	24. xii H	3. i O						
V. Th. 734	D	9. xii Δ	12. xii O	18. xii H	20. xii H	3. i O						
B. C. 792	D	28. xii Δ	2. i Δ	3. i Δ	7. i Δ ⁽¹⁾	9. i Δ	21. i Δ	28. i Δ	5. ii O			
					7. i H ⁽¹⁾			8. ii O	11. ii O			
A. 735		10. xi O	12. xii O	13. xii O	14. xii O					Turned out to be scarlet fever		

TABLE III.

TESTS OF VIRULENCE OF THE CULTURES ISOLATED.

(A) Acid forming bacilli identical with one or other of the types of diphtheria bacillus.

(a) *Virulent.*

[illegible]

M.C.	47a	D	13 . xi	215 200	0.1 0.05	2 days "	T SS T +	+	N N N	N N N	N N N	N N N	} Instance of apparent increase of virulence of culture due to adaptation to laboratory media. 12 days elapsed between the inoculations } do. do. 3 days' interval (1) With antitoxin
"	"		"										
M.Cl.	723	D	1 . xii	250 220 250 220 220 220	0.1 0.1 0.1 0.25 2.0 (1) 0.15	2 days " " " " "	T SS SS SS SS SS	N + N N N +	N N N N N N	N N N N N N	N N N N N N		
"	"		"										
"	"		"										
"	"		"										
"	723b		20 . xii	220 220	0.15	"	SS SS +	N N +	N N N	N N N	N N N	N N N	
V.Th.	784	D	18 . xii	250	0.1	2 days	SS +	+					
G.D.	714a	D	11 . xii	250 250 270	0.1 0.05 2.0 (1)	2 days " "	SS SS SS	+					Culture during convalescence Small patch of necrosis (1) With antitoxin
"	"		"										
"	"		"										
R.G.		D	20 . xii	200 200	0.1 0.1	2 days "	SS SS +	T N +	T N N	N N N	N N N	N N N	
"			"										
Mrs W.	778		21 . xii	220	0.1	2 days	SS +	+					
P.	790	D	27 . xii	260	0.1	2 days	SS +	+					
B.C.	792	D	28 . xii	220	0.1	1 day	T +						
"	792b		2 . i	180	0.1	2 days	MS +	+					
"	792c		7 . i	200	0.1	1 day	LS +	+					
A.M.	788	D	27 . xii	270	0.1	2 days	SS +	+					

(b) *Not virulent.*

A.Mt.	260		30 . x	0-1 250 1-0 180 2-0	2 days ,, ,,	T SS SS T	N T T	Z N Z T	Culture during convalescence
A.G.	721	D	30 . xi ,, ,,	265 210 240 2-0	3 days 2 days ,,	SS SS SS T	N T N	N Z Z Z	
B.	782	I	24 . xii ,, ,,	185 220 200 2-0	2 days ,, ,,	T T T	N N N	N Z Z	
Mrs C.	793		28 . xii ,,	290 230	2 days ,,	T N	N N	N Z Z	

TABLE III. (*cont.*)

(B) Short bacilli of the Löffler-Hofmann type which do not produce acid in glucose broth.

[illegible]

TABLE III. (cont.)

List number and initials of person from whom culture was obtained	D = a clinical case of diphtheria	Date of swab	Weight of guinea-pig grms.	Quantity and age of culture injected		Result : $\left\{ \begin{array}{l} N = \text{No local swelling.} \\ SS = \text{Small swelling.} \\ T = \text{Trace.} \end{array} \right.$											Remarks
				gms.	c.c.	1	2	3	4	5	6	7	8	9	10	11	
F.W. 541		3. xi	340	2.0	7 days	SS	T	N	N	N	N	N	N				
C.S. 395		1. xi	500	2.0	7 days	SS	N	N	N	N	N	N	N				
H.H. 443		2. xi	370	2.0	7 days	T	T	N	N	N	N	N	N				
E.M. 558		6. xi	190	1.0	4 days	N	N	N	N	N	N						
A.Mn. 293	D	30. x	200	1.0	4 days	N	N	N	N	N	N	N	N	N			
S.Mt. 304a		8. xi	200	1.0	8 days	T	N	N	N	N	N	N	N				
A.Mt. 260b		8. xi	200	1.0	2 days	N	N	N	N	N	N	N	N	N			
E.R. 517		3. xi	210	1.0	2 days	T	N	N	N	N	N	N	N	N			
P.T. 404		1. xi	200	2.0	12 days	T	N	N	N	N	N	N	N	N			
H.M. 162a	D	27. x	230	1.0	2 days	T	N	N	N	N	N	N	N	N			
M.W. 84		24. x	210	1.0	2 days	T	N	N	N	N	N	N	N	N			
R. 560		6. xi	240	1.5	3 days	T	N	N	N	N	N	N	N	N			
F.B. 344		31. x	225	1.5	3 days	T	T	N	N	N	N	N	N	N			
A.H. 505		3. xi	240	1.5	3 days	T	N	N	N	N	N	N	N	N			
M.M. 538		3. xi	420	1.5	3 days	SS	T	N	N	N	N	N	N	N			
N.H. 251		29. x	230	1.5	2 days	T	T	N	N	N	N	N	N	N			
S.Cl. 511		3. xi	370	1.5	2 days	SS	T	T	N	N	N	N	N	N			
B.Cl. 514		3. xi	370	2.0	2 days	T	N	N	N	N	N	N	N	N			

E.Cl. 512	3. xi	470	2.0	2 days	SS	T	N	N	N	N	N	N							
E.N. 391	1. xi	260	2.0	2 days	T	T	N	N	N	N	N	N							
G.T.R. 25	17. xi	220	2.0	2 days	T	N	N	N	N	N	N	N							
M.E.R. 26	17. xi	240	1.5	?	SS	T	T	N	N	N	N	N	N						
G.M. 672	14. xi	180	1.0	2 days	T	N	N	N	N	N	N	N	N	N				N	
G.H. 445	2. xi	180	1.0	2 days	N	N	N	N	N	N	N	N	N	N				N	
A.C.R. 28	17. xi	220	0.5	2 days	T	T	N	N	N	N	N	N	N	N					
Jk.S. 82a	24. x	180	1.5	2 days	T	T	T	T	T	T	T	T	T	T					
" 82d	10. xi	230	1.0	2 "	N	N	N	N	N	N	N	N	N	N	N				
G.D. 324	31. x	180	1.5	2 days	T	T	N	N	N	N	N	N	N	N					
J.B. 499	3. xi	200	2.0	4 days	T	N	N	N	N	N	N	N	N	N					
" "	"	200	2.0	4 "	SS	T	N	N	N	N	N	N	N	N					
D.Cl. 733	8. xii	250	2.0	2 days	SS	N	N	N	N	N	N	N	N	N					
G.D. 244a	D	210	2.0	2 days		T	N	N	N	N	N	N	N	N				Culture during convalescence	
A.L. 769	D	220	2.0	2 days		T	N	N	N	N	N	N	N	N				Culture during convalescence	
W.R. 799	28. xii	200	2.0	2 days	SS	T	N	N	N	N	N	N	N	N					
L.C. 798	28. xii	205	2.0	1 day	SS	T	N	N	N	N	N	N	N	N					
A.C. 806	31. xi	250	2.0	2 days	SS	N	N	N	N	N	N	N	N	N	N				
M.C. 723c	D	250	2.0	2 days	SS	T	N	N	N	N	N	N	N	N				Culture during convalescence	
R.A. 746a	28. xii	180	2.0	2 days	T	N	N	N	N	N	N	N	N	N					
R.W. 17c	21. xii	200	2.0	2 days	N	N	N	N	N	N	N	N	N	N	N				

Table III. gives a complete list of the guinea-pig inoculations which were made with the isolated cultures. Except in a few instances an autopsy was made on the animals which died, and all those which had been injected with *acid producing bacilli* were found to have the characteristic signs which follow the injection of diphtheria bacilli or their toxins into the subcutaneous tissue of guinea-pigs.

Conclusions.

1. Experience of the outbreak in Cambridge gave no reason for thinking that the pseudo-diphtheria bacillus is other than perfectly innocuous to man.

2. The relationship between the pseudo-diphtheria and the diphtheria bacillus remains undecided. Even though it should be definitely established that by laboratory procedures the former can be converted into the latter, it must yet be shown that the change occurs under natural conditions.

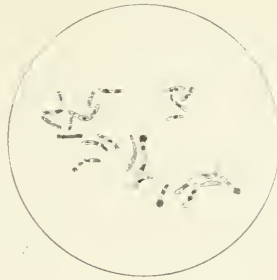
3. The frequent presence of the pseudo-diphtheria bacillus should not be allowed to weaken our efforts to detect and isolate those who harbour the virulent bacillus.

4. The principal means of combating diphtheria are, after the isolation of persons actually sick, the detection of those who go about apparently in good health carrying with them the diphtheria bacillus, and the isolation of such persons, and of convalescents from the disease until diphtheria bacilli can no longer be cultivated from them. No doubt the satisfactory isolation of healthy persons who carry about the bacillus will often prove impracticable. In such cases the infectious persons should be warned that they are a danger to others, and instructed to take certain precautions, which need not be detailed here. In the case of children isolation will usually be practical, and experience among the poorer classes at Cambridge has shown that parents can usually be brought to consent to the removal of their children to an isolation home.

5. With increasing confidence in the bacteriological test on the part of the medical profession and of the general public, such measures will be much facilitated. But this confidence will not be forthcoming until bacteriological examination distinguishes clearly between the diphtheria and the pseudo-diphtheria bacillus.

I. *Diphtheria bacilli, virulent, acid formers.*258^a

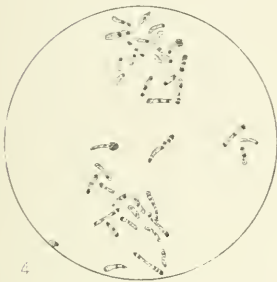
B.C. 792. 28. x11.



B.C. 792. 3. 1.



Mrs M. 250.



M.Cl. 723. 1. x11.



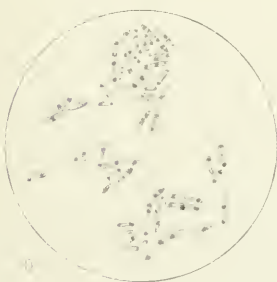
M.Cl. 723. 17. x11.



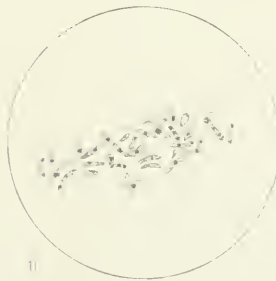
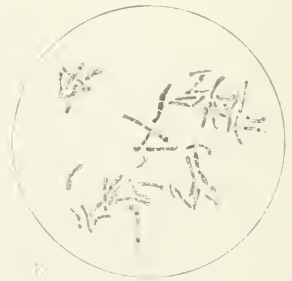
M.Cl. 723. 20. x11.

M.Cl. 723. 1. x11.
broth, 3 days.M.Cl. 723. 1. x11.
Glucose broth, 24 hours.

G.Gd.

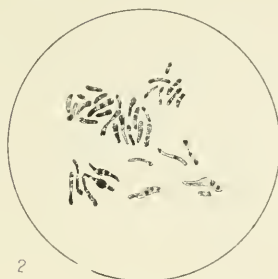


R.G. 768.

R.G. 768.
Serum Subculture, 24 hrs.R.G. 768.
Serum Subculture, 21 hrs.

Diphtheria bacilli (continued)

V.Th. 734
3 days.



V.Th. 734
Serum Subculture, 2 days.



G.N. 1. a. Chr. Fibrinous Rhinitis.
Broth, 2 days.



V.Th. 734.
Glucose broth, 2 days.



V.Th. 734.
Sugar-free broth, 2 days.



G.D. 714.
Glucose broth, 3 days.



G.D. 714.
3 days.



G.D. 714.
Sugar-free broth, 24 hours.



G.D. 714.
Glucose broth, 24 hours.

II. *Non-virulent diphtheria bacilli, acid formers.*



A.G. 721. 3 days.
During convalescence from diphtheria.



A.G. 721.
Sugar-free broth, 24 hrs.



A.G. 721.
Glucose broth, 24 hrs.

Non-virulent diphtheria bacilli (continued).

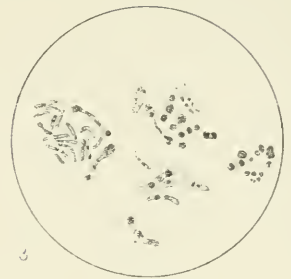
258



Mrs. C. 793.



B. 782.
Sugar-free broth. 8 days.

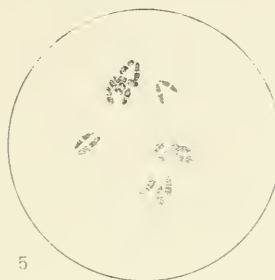


B. 782.
Sugar-free broth. 24 hrs.

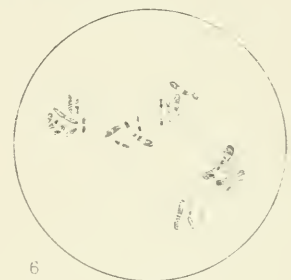
III. Pseudo-diphtheria bacilli, non-acid formers.



G.G. 375.
Glucose broth. 2 days.



D.Cl. 733.



D.Cl. 733.
Glucose broth 2 days.



M.W. 777.



G.D. 754.
3 days.



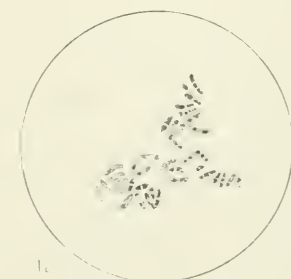
R.A. 796.
Glucose broth. 24 hrs.



A.L. 789.
Sugar-free broth. 2 days.



M.Cl. 723. 3 days.



M.Cl. 723. 3 days.

4th Culture from this case, the previous three
being diphtheria bacilli (see Table II.).

6. The prophylactic use of antitoxin during the outbreak of diphtheria at Cambridge proved of great value. It ought always to be accompanied by bacteriological examination, lest by the suppression of symptoms of disease it be the means of preventing the isolation of those who would have fallen ill, and of allowing them to go free and distribute infection. The need of this precaution is strengthened by the fact that the few persons who continued to harbour the diphtheria bacillus for a long time were among those who in consequence of a timely dose of antitoxin, or because they were naturally insusceptible, were little or not at all affected. It seems probable that an active inflammatory reaction is instrumental in expelling the bacilli, which are consequently more apt to persist if this is prevented.

EXPLANATION OF PLATES.

The initials and numbers placed below the drawings correspond to those in the tables, so that various particulars about the culture represented can be ascertained.

In one case no number is given, the preparation being from a case not included in the tables.

Except when stated otherwise the drawings were made from the original serum culture after 24 hours' incubation.

When drawings of more than one culture taken at different times from the same person are given the date of each is given for reference to Table II.

The drawings ($\times 1000$) were made with a Camera Lucida, a Zeiss oil immersion $\frac{1}{2}$ in. Objective, and Eyepiece C.

THE SHANGHAI PASTEUR INSTITUTE.

By ARTHUR STANLEY, M.D., B.S. Lond., D.P.H.

Health Officer of Shanghai.

THE idea of having the means of applying the Anti-rabic treatment of Pasteur in Shanghai, China, had its origin in the marked incidence of rabies among the numerous dogs of the Shanghai district and the consequent large mortality from this disease. The local condition was the more marked because of the short incubation period observed in Shanghai both in the human subject and in the rabbit inoculated from the rabid dog, being seldom much over a month in man and rarely over two weeks in rabbits inoculated subdurally.

As the Shanghai laboratory was the first in British hands to offer to the public the advantages of Pasteur's treatment it may prove interesting to describe the beginnings of this institution as a part of the work of the Health Department of the Shanghai Municipal Council. Notwithstanding the comparative isolation of Shanghai from Western civilisation an efficient Public Health Service has been organised as a necessary branch of the local government of the foreign settlement. This includes a well equipped laboratory affording every facility for original investigation.

In 1898 after making inoculations in the Shanghai laboratory of rabbits from dogs suspected of rabies, I worked at Kitasato's laboratory in Tokio, Japan, receiving there myself the anti-rabic inoculation for prophylactic purposes and procuring the brain of a rabbit, preserved in glycerin, which had died of the intensive virus of rabies, with which to commence the series in Shanghai. On returning to my own laboratory I inoculated two rabbits subdurally with this virus (then fourteen days old) but obtained no result and even after several trials it proved innocuous¹. Another source of virus was therefore sought. Dr Simond,

¹ It is possible that the glycerine used for preserving the brain may have been impure or the different breed of rabbits used may have had something to do with it—the animals used by Kitasato being immense creatures with curly hair, while the Shanghai rabbits were mostly small albinos bred in the laboratory, aged about three months and scarcely weighing more than one kilo.

the Director of the Saigon Pasteur Institute, kindly sent me in February 1899 the brain of a rabbit, which had died sixteen days previously, the incubation period having been seven days. The brain was preserved in glycerin in a sealed tube. At about the same time another brain from a rabbit presenting symptoms of rabies on the seventh day and dying on the tenth day was received through the post from Kitasato in Tokio. Both of these brains proved effective and the series (1) from the Saigon Pasteur Institute, (2) from Kitasato's laboratory in Tokio, after a preliminary lengthening of the incubation period to the ninth day or thereabouts assumed one varying between six and eight days.

TABLE I.

Showing the identity of incubation period and duration of the disease in rabbits inoculated with intensive virus of rabies from two distinct sources.

The figures relate to the first 24 rabbits inoculated of each series.

Rabbit No.	Virus—Kitasato (Tokio)		Virus—Simond (Saigon)	
	Incubation in days	Day of death	Incubation in days	Day of death
1	9	13	9	13
2	10	15	9	12
3	8	11	8	10
4	8	10	8	10
5	7	12	7	10
6	11	14	8	10
7	7	10	8	12
8	8	10	8	10
9	7	9	16	19
10	10	14	8	11
11	10	15	7	9
12	9	10	8	13
13	8	10	8	14
14	8	12	6	11
15	7	11	7	10
16	7	10	7	12
17	7	11	7	11
18	12	17	6	9
19	7	11	7	11
20	7	11	7	10
21	7	12	7	13
22	8	12	8	12
23	7	10	7	12
24	6	9	7	13
Average	8.1	11.6	7.8	11.6

It will be seen from the tables that the incubation and day of death average out much the same.

TABLE II.

Incubation period of 379 rabbits inoculated 1899—1900				
Number in which incubation was	10 days or more			18
" " " "	9 days			26
" " " "	8 days			95
" " " "	7 days			185
" " " "	6 days			55

TABLE III.

Duration of symptoms in 379 rabbits inoculated 1899—1900	
6 days	12
5 "	36
4 "	45
3 "	123
2 "	137
1 "	26

Two rabbits after manifesting symptoms of rabies recovered.

The Shanghai Pasteur Institute after six months' preparation was opened to the public for treatment in March 1899 and the series has been continuously maintained, some 379 rabbits having been inoculated.

Up to the present date seventeen persons have received the treatment, including five for prophylactic purposes. In six the dog which caused the bite was proved rabid by inoculation in the laboratory. In one the dog died of undoubted rabies certified by a qualified veterinary surgeon. In five the dogs were not heard of after the bite, so that there was only presumptive evidence of rabies. Among those treated there were two deaths, one from undoubted rabies 33 days after the bite and another presumably from rabies (the case was not seen by a medical man) 27 days after the bite, but it is a significant fact that in each of these cases the treatment had been interrupted; in one case the patient (a Chinese) not presenting himself for treatment for five days and in the other, on account of alcoholism, for one day during the

treatment. Moreover these were the only two of the seventeen in which the treatment had been interrupted. The history of these two cases is as follows:

Case I. A Swedish Policeman was bitten slightly in the wrist while attempting to catch a dog. The animal was subsequently shot, though not suspected of rabies. On account of its having bitten the man a rabbit was inoculated from the dog. The rabbit presented symptoms of rabies on the 12th day and died on the 16th day. The man began the simple Pasteur treatment on the day following the bite, beginning with the cord dried 14 days and diminishing one each day up to a cord dried three days. The man got drunk and did not come for treatment on the 12th day. The treatment lasted 20 days, the latter part consisting of a repetition of cords dried five, four and three days respectively. He presented symptoms of hydrophobia exactly one month after the bite and died two days afterwards, 33 days after receiving the bite.

Case II. A Chinese small boy was bitten through the nose by a dog whose brain inoculated into rabbits produced rabies after an incubation period of 13 days. The intensive treatment was given, a cord as strong as the seventh day of drying being injected on the fourth day of treatment. The child did not come for treatment from the 11th to the 17th day. The treatment lasted 22 days including the five days during which the treatment was interrupted, the strongest cord given having dried three days. The child is reported to have died 27 days after the bite. It was not seen by a medical man, but as the child appeared well when I last saw him five days previously, it is probable that he died of hydrophobia having a remarkably short incubation.

The comparative fewness of cases receiving the Pasteur treatment in Shanghai is explained by the prejudice of the Chinese against Western Medicine. However, five Chinese received the anti-rabic injections, including four of my Chinese laboratory assistants.

Methods employed at the Shanghai Pasteur Institute.

The method used in the Shanghai laboratory is modified by the comparative scarcity of cases seeking treatment. In order to reduce the manipulations and the animals used, to an effective minimum, it has been found that one rabbit developing rabies every second day, suffices for a descending series of dried cords beginning on any given odd or even day, as follows:

TABLE IV.

Method used for obtaining a series of dried cords with a minimum of rabbits inoculated.

Day of month	No. of days of drying of cord									
1	1									
2	2									
3	3	1								
4	4	2								
5	5	3	1							
6	6	4	2							
7	7	5	3	1						
8	8	6	4	2						
9	9	7	5	3	1					
10	10	8	6	4	2					
11	11	9	7	5	3	1				
12	12	10	8	6	4	2				
13	13	11	9	7	5	3	1			
14	14	12	10	8	6	4	2			
15		13	11	9	7	5	3	1		
16		14	12	10	8	6	4	2		
17			13	11	9	7	5	3	1	
18			14	12	10	8	6	4	2	
19				13	11	9	7	5	3	1
20				14	12	10	8	6	4	2
21	13	11	9	7	5	3	1
22	14	12	10	8	6	4	2
23		13	11	9	7	5	3
24		14	12	10	8	6	4
25	13	11	9	7	5	3
26	14	12	10	8	6	4
27		13	11	9	7	5
28		14	12	10	8	6
29	13	11	9	7	5
30	14	12	10	8	6
1	13	11	9	7
2	14	12	10	8
3	13	11	9
4	14	12	10
5	13	11
6	14	12
7	13
8	14

By these means on any given day a series of dried cords for injection into a patient can be commenced, beginning with a cord which has dried 13 or 14 days, and diminishing by one day of drying each day. It is usual however in Shanghai, where, on account of the short incubation period of street rabies, almost every case is treated by the more intense method, to give on the first day emulsions of cords dried 13, 11 and 9 days respectively, and on the second day those dried 12, 10 and 8 days, the series being rapidly ascended to cords dried 7 days.

In order to allow for contingencies of lengthened incubation, or of failure to contract rabies, an extra rabbit over and above the series is inoculated weekly. Thus the inoculation of some 235 rabbits yearly suffices for the maintenance of the series and for the treatment of at least ten cases a day. It is not necessary for a rabbit to die precisely every second day, for if killed after the symptoms of rabies are developed, the brain is equally virulent, as is also the brain of a rabbit dead of rabies preserved for a few days in ice.

The series of dried cords used in the cases first treated, was the simple one beginning with a cord dried 14 days, followed on each succeeding day by a cord dried a day less. When however the street rabies was found to be of a particularly virulent type it was thought expedient to use a more intensive treatment even for ordinary cases, and the series now employed is given in the following table.

In two cases the injections were, with the happiest results, carried down to cords dried only one day, and in two other cases in which the treatment was commenced 14 days after the bite, the series was started by injecting a 7 day cord.

TABLE V.

Series of cords used for anti-rabic treatment.

Day of treatment	Day of drying of cord		
	For prophylactic purposes	For small bites on extremities	For deep bites, bites on head and face & where cases come under treatment late
1	14	14 and 12	13, 11 and 9
2	13	13 „ 11	12, 10 „ 8
3	12	10 „ 8	7
4	11	9 „ 7	6
5	10	6	5
6	9	5	4
7	8	4	3
8	7	3	6
9	6	6	5
10	5	5	4
11	4	4	3
12	3	3	6
13		4	5
14		3	4
15			3
16			6
17			5
18			4
19			3
20			4
21			3

The method used at Kitasato's laboratory in Tokio (where the number of cases is also small, owing to the rarity of rabies in Japan) differs from ours in that emulsions of the cords of different days' drying are made, and kept up to three days in ice for use if called for—*e.g.* a cord dried say 14 days is made into an emulsion on that day and, kept in ice, may be given three days hence as an emulsion of a 14th day dried cord. I prefer, however, to use freshly prepared emulsions of the dried cord because they undoubtedly become altered by keeping, and freshly prepared emulsions are less liable to contamination by external organisms. I have found that the cords though removed under strict asepsis and manipulated entirely in sterilised vessels, more frequently than not, when inoculated on agar, give rise to bacterial growth. At the Saigon Pasteur Institute cords are dried the requisite number of days to form the series, and preserved in glycerin in sealed tubes. These tubes may be sent through the post, whereby treatment can be carried out at a distance from the mother institution.

Inoculation of Rabbits.

In the beginning sub-dural inoculation was practised, by cutting down on the bone, reflecting the periosteum and trephining near the middle line at the point where the line joining the posterior margin of the orbits crosses it. The circle of bone having been removed the injection was made with a hypodermic syringe immediately below the dura mater. This method was later replaced by that of intra-cerebral inoculation described by Leclainche and Morel¹ which has proved simpler and quicker and quite as effective. The hair of the top of the head and between the eyes and ears is cut as short as possible with scissors. The animal is fixed on a rabbit board having a mouth-piece and the shaved portion is sterilised with a 10% solution of lysol in alcohol, which is allowed to remain on for a quarter of an hour. No anæsthetic is given, as it was found that very little pain was inflicted. No sign of pain was given by the animal except when, at the end of the operation, sutures were passed to draw the edges of the skin incisions together. The suturing has been replaced by merely approximating the edges of the skin incision with collodion, and, apart from the discomfort of being fixed on the board in an unnatural position I am of opinion that very little pain is inflicted. The skin, which is very thin, is incised to the bone for a distance of 1.5 cm. about 2 mm. from the median line, the middle of the incision being on a line joining the posterior margins of the orbits. The periosteum is reflected from an area of bone sufficiently large to permit of the application of a drill 2 mm. in diameter. A minute hole is made in the cranium just large enough for the passage of a hypodermic needle. The needle is thrust gently in a direction forwards and outwards to the depth of

¹ *Ann. de l'Inst. Pasteur*, 1899, vol. XIII., p. 513.

1 cm. and 0.1 c.c. (about 2 drops) of emulsion is injected. If much more than this is injected the rabbit dies with Jacksonian convulsions, contractures and coma. The periosteum is now replaced over the point of entrance to the brain cavity, and the skin surfaces apposed and sealed together with collodion. If this seal is imperfect, and the escape of cerebro-spinal fluid permitted, the animal usually dies on the following day. The effectual sealing of the wound is important. The rabbit is now marked with the day of the month on its back with a solution of fuchsin in alcohol and the particulars of source of virus and date of inoculation entered on a sheet suspended over the hutches where the inoculated rabbits are kept.

The virus for inoculation of the rabbits is prepared by taking a fragment of the bulb of the preceding rabbit dead of intensive rabies and grinding it to a cream in a small porcelain mortar, sterilised by boiling in plain water. Any fibrous piämater is removed. A few drops of sterile normal saline is added to make the emulsion of such a consistency as can be taken up by a hypodermic syringe. The kind of *hypodermic syringe* is of some importance, and the only one which has given complete satisfaction, by being easily sterilisable by boiling and never getting out of order, is the all-metal one with a steel piston and platino-iridium needles. The steel piston requires lubrication, sterile castor-oil being used. By these means rabbits rarely die of anything but rabies after the operation, and very seldom does rabies fail to supervene.

Preparation of Cords for Use in Anti-rabic Treatment.

The inoculated rabbits begin to show symptoms of rabies on the sixth to eighth day, refusing food, and hanging down the head. They are sometimes excitable, the stage of excitation being followed by paralysis of the limbs, the onset of which can be most easily tested by the strength with which a lateral push is resisted. Finally the limbs give way, the animal lies helpless and there is usually diarrhoea.

The dead rabbit is placed on a galvanised iron tray and its legs tied to rings at the four corners. The head and back are sterilised by alcoholic lysol (10 %) allowed to remain on for half an hour. The instruments necessary are sterilised by boiling in plain water, namely, three sets of forceps and scalpels, with scissors and bone-forceps angled on the flat. The skin is first reflected and then the cranium and vertebral column are freed from muscle. With bone-forceps, working from behind forwards, the cord and brain are exposed by cutting through the bony casing alternately on either side. The cord is raised from its bed by passing the end of the blunt-pointed scissors rapidly along each side so as to detach the spinal nerve junctions. A fine silk ligature is passed round the cord in the lower dorsal region and the cord cut through at this point and near the bulb, so as to leave a length of about 10 cm. hanging from the ligature. This is suspended in an ordinary narrow-mouthed stoppered bottle which has been sterilized and contains some freshly dehydrated caustic potash, soda or calcium chloride. The silk ligature passes out between the stopper and the neck of the bottle and by this it is manipulated. The date on which the cord is placed in the drying bottle is marked clearly by means of alcoholic fuchsin with a brush on the outside of the bottle. This is then placed

in a dark incubator provided with a capsule regulator, the temperature being kept constantly at 23° C. by means of hot water in winter and ice in summer. The apparatus used by me was made by Hearson, and, though the details of workmanship are careless, the regulator works well.

To prepare the emulsion of the cord for anti-rabic treatment 1 cm. of cord is cut off and triturated dry in a small porcelain mortar such as can be conveniently boiled bottom upwards in a small steriliser. When completely broken up 5 c.c. of sterile normal salt solution are added, and a fine emulsion made by the pestle. The dose of this is 1 c.c., the equivalent of 2 mm. of cord.

STUDIES IN RELATION TO MALARIA.

II.

THE STRUCTURE AND BIOLOGY OF ANOPHELES

(Anopheles maculipennis).

BY GEORGE H. F. NUTTALL, M.A., M.D., PH.D.,

University Lecturer in Bacteriology and Preventive Medicine, Cambridge;

AND ARTHUR E. SHIPLEY, M.A.,

*Fellow and Tutor of Christ's College, University Lecturer on the Advanced
Morphology of the Invertebrates, Cambridge.*

*(From the Pathological and Morphological Laboratories of
the University of Cambridge.)*

Continued from page 77.

III. THE PUPA.

External Structure.

THE larva about to turn into a pupa comes to rest, the thoracic region becomes swollen, and the pupa gradually issues through a dorsal slit in the larval cuticle, which is ultimately thrown off with all its exterior chitinous appendages as well as those parts of the spiracles etc. which are superfluous.

A favourable specimen threw some light on the change from larva to pupa, it was in fact a very young pupa which had half cast off its larval skin and was in the act of freeing its tail. The collar round the base of the head of the larva had split in the middle dorsal line and a gape had appeared, this gape was continued forward, shaped like a Y,

along the lines of the posterior sides of the diamond-shaped area already described on the head, in this way the centre of the upper surface of the head projected as a kind of lid or flap. The skin of the body had also split along the back and the young pupa was just freeing its tail from the cast-off skin. This pupa measured 6.5 mm., of which the head and thorax measured 2 mm. As the pupa matures the head and thorax increase in size up to about 3 mm. in length, the abdomen looks relatively small, more like a tail, and is bent round along the ventral surface of the sac.

During the last larval stadium the various pupal organs are being formed. When the larva gives rise to the pupa the head and thorax are already in their "sac," the respiratory trumpets are there, the tail fins, mouth parts, and limbs are enclosed in their sheaths, the former showing no relation to the mouth parts of the larva.

Whereas the larva breathed through two spiracles which ran along the back, gradually increasing in size and terminating in two stigmata at the posterior extremity (see p. 64), the whole respiratory system of the pupa is reversed, together with the position of the insect, when it assumes the pupal form. The insect now breathes through two respiratory trumpets issuing laterally from the anterior dorsal surface (Plate II, fig. 10), these forming apparently the only external openings. The insect does not feed during the pupal stage, during which it only undergoes its metamorphosis into the imago.

The pupa is a tadpole-looking object, but the comparison would be more correct if we imagine the tadpole has its tail flattened in a horizontal plane and folded under the body. The whole of the head and thorax is enveloped in a thin and semitransparent membrane, within which the various appendages (and even the scales upon them) can be seen coiled up in a symmetrical manner. We shall call that part of the body included in this membrane the "sac."

The head is folded down upon the breast. The most conspicuous organs of the body are the eyes, which are already black and consist of an increasing number of ommatidia. Just anterior and above the eye the antennae emerge from the head and are folded backwards, crossing the upper part of the head, and passing backwards across the origin of the three pair of limbs. They then lie parallel and between the posterior pair of legs and the anterior edge of the wings.

As the pupa increases in age it perceptibly darkens, and the darker parts are usually first apparent at the ends of the appendages and on the wings. The pupa is occasionally green. We have seen quite an

old specimen in which all the internal parts were coloured green and shone through the transparent cuticle.

The mouth parts are very long and are coiled symmetrically. The maxillary palps do not reach so far forward as the base of the anterior pair of legs, but their great length is provided for by the palps being sharply bent back and their curving forward again in a somewhat S-shaped manner.

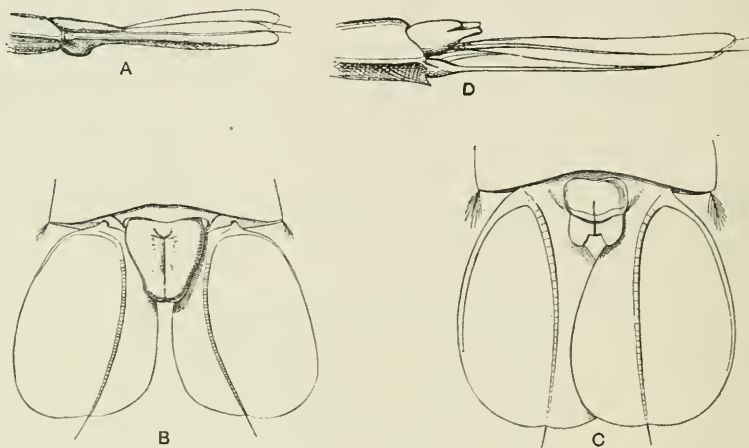
The labrum, mandibles, maxillae, labium and hypopharynx all pass backward like the trunk of an elephant curled in, in the median plane. On each side of this middle organ lie the legs, all passing backward and converging inwards, at the hinder end. As the legs are too long for the case they bend gracefully upwards and forward in a quite symmetrical manner. The sac extends ventrally as far as the division between the 2nd and 3rd post-thoracic segment and hides the sterna of the anterior abdominal segments, but dorsally the terga of the last thoracic and first two abdominal segments are quite distinct.

The breathing trumpets have their origin from two very stout and conspicuous tracheae which run backward and inwards parallel with the base of the wing; at its inner end this trachea is slightly constricted just where it passes the integument to open into the external organ or trumpet. This is not really trumpet-shaped, but more like a cornucopia of paper, that is, on the face directed inwards there is a V-shaped slit of very graceful form. The edges of this slit are, when the pupa is breathing, supported by the surface film. The edges sometimes overlap one another for a little near the base, and the surface of the whole trumpet is marked out into very delicate spindle-shaped areas.

Wedged into the space between the wings is the tergum of the first abdominal segment. The ventral part of this segment is included in the sac and does not stand clear. The second abdominal segment is free, and this is followed by six other segments all more or less resembling one another. The eighth segment bears posteriorly two large flaps or fins measuring usually about .8 mm. in length. Each fin has a chitinous ring at its base and a weakly chitinized transversely striated bar runs through about the centre of the fin as a strengthening skeleton, it thins out at the periphery and terminates in a single hair. The right fin overlaps dorsally the left. (Fig. C.)

The ninth segment bears a pair of blunt processes within which the gonapophyses develop. These processes are situated in front of and between the fins, below and in front of the anus. The processes are much larger and of a different form in the male pupa, so that it is possible

even on naked-eye examination to determine the sex of the living pupa by viewing it through the sides of the aquarium. The accompanying figures serve to show this difference. Figures *A* and *B* represent these structures in the male (lateral and ventral views), *D* and *C* the corresponding parts in the female pupa. Figure 10 on Plate II. represents a male pupa viewed from the side, *x* representing the processes which contain the gonapophyses.



From the second to the eighth the segments have much the same structure. The narrow chitinous tergum of the larva has increased and forms a large plate, stretching from side to side and covering from before backward three-quarters of the area. At each side the chitin of the tergum is thickened and darkened, and at the posterior angle it is produced into a stout, backwardly directed hair. The thickening gives the pupa sharp sides to its body, the cross section of which is no longer, as in the larva circular, but somewhat spindle-shaped. Posteriorly the terga bear four symmetrically arranged branched hairs which project back over the soft skin which forms the posterior fourth of the segment uncovered by the tergum. Anterior to these is a pair of similarly three or four times branched hairs, and some long single ones. The tergum does not cease laterally at the thickened sharp lateral edge but bends under and forms a small plate increasing in width from before backwards. The inner posterior angle of this plate bears a hair.

When viewed sideways the pupa of *Anopheles* presents a comparatively smooth dorsal outline, but in *Culex* the edge where each tergum

joins posteriorly the soft integument which unites it with the succeeding tergum stands out as a ridge, and the dorsal outline presents a series of salient angles. We might add that Howard¹ (1900, p. 40) draws attention to the fact that the pupa of *Culex* assumes a more vertical position in the water than that of *Anopheles*, and that its respiratory trumpets are not so broad terminally.

The sterna are of some size, not so broad as the terga but stretching over the middle three-quarters of the ventral surface, anteriorly rather rounded, they are posteriorly sharply cut where they unite with the soft intersegmental membrane. In the older pupae they bear numerous rows and patches of minute hairs, symmetrically arranged, these hairs point backwards and outwards, and the central ones on the eighth abdominal segment point inwards and are longer than the others.

Similar symmetrically arranged hairs also pointing backwards and outwards are found on the terga. Since each hair articulates by a deeply pigmented base, there is an increase in the coloration with the appearance of these hairs.

As the pupa increases in size more hairs appear and additional chitinizations arise in the skin, a median small plate occurs behind each tergum, and this is produced laterally into two side dorsal plates which look as though they were perforated.

Just in front of the anterior abdominal tergum, which as has been said is dovetailed into the dorsal part of the thorax, is another small tergum which is continued on each side into a flattened process which bends in and is concealed under the wings. This would seem to be the segment which bears the halteres. It and the first abdominal segment are hollowed out, bent in as it were like the small of the back. This hollow is the surface which lies uppermost when the pupa is in its usual position floating at the surface of the water, and the creature is maintained in this position by a pair of those conical shaped hairs which played the same part in the larva. The hairs are stalked and bear secondary hairs, like the ribs of an umbrella turned inside out, but in the pupa the secondary hairs are filiform, not flattened, as they are in the larva. They are black and do not form a complete cone, one quarter being absent.

The pupa, until about the time when it gives rise to the fly, floats quietly at the surface, breathing through its respiratory trumpets. The trumpets slightly indent the film, over which at times the dorsal surface

¹ See Bibliography, p. 75.

of the pupa may protrude. When disturbed the pupa shows great activity, and apparently purpose, in its movements, as is seen when the attempt is made to capture it by means of a pipette. It darts rapidly with a series of quick intermittent strokes of its muscular abdomen to the bottom of the vessel. On account of its great buoyancy it again quickly rises passively to the surface as soon as it ceases to move. It advances tail first, and owing to the motion of the abdomen being apparently limited, that is dorso-ventral, it only moves downward, counteracting the buoyant action of the air vesicle and the air contained in the respiratory trumpets and tracheae which keep the anterior portion of the body uppermost. The effectiveness of the tail as a swimming organ is materially increased by the broad flaps with which it terminates. When extended the abdomen assumes the position which the abdomen of the fly occupies in relation to the thorax. This is best seen in dead pupae, where owing to the relaxation of the parts the abdomen becomes extended in a straight line. Locomotion is effected by means of powerful muscles situated ventrally within the abdomen. Besides the air in the respiratory trumpets and tracheae there is a considerable reservoir of air at the posterior end of the sac. This air is ventral, but extends some distance up each side of the body. It acts as a very efficient float, keeping the animal right way upwards and enables it to float up to the surface the moment it ceases swimming. The position of the pupa in rising to the surface is determined by the air vesicle, the bubble of air being visible through the pupal covering, especially of young pupae. Owing to its great buoyancy and lack of weight so to speak in its "keel," combined with irregularities in locomotory movements, the pupa at times floats sideways beneath or near the surface, moves sideways and downwards etc., but soon rights itself when it ceases to struggle.

The movements of the pupa are so powerful that if placed in a thin glass vessel containing water it produces an audible sound by striking against the walls of the vessel. Pupae which have retreated from the surface frequently show two minute air-bubbles at the ends of their trumpets, these bubbles bursting when they come in contact with the surface again.

The surface of the sac becomes darker with age and develops numerous small hairs, and a fairly conspicuous bunch of longer hairs, which arise just inside the base of the respiratory trumpet and are closely adpressed to the surface of the pupa. All along the top of the head, stretching from the "forehead" back between the respiratory

trumpets almost to the end of the "sac" is a special area bounded by two slightly elevated lateral ridges and divided into two equal halves by a strongly marked ridge. The areas each side of this latter are faintly marked out into small squares.

Duration of pupal stage.

The pupal stage observed in 37 insects kept isolated and separately observed, was found to last from 3 to 4 days. The pupae were removed from tanks immediately after transformation and placed in bottles containing clear water. The temperature at which the pupae were kept was found to exert a considerable influence upon the rate at which metamorphosis took place. Three pupae kept at 23.7° C. developed on the fourth day. Twenty-three kept at an average temperature of 25.2° gave rise to flies on the third day. Two of the latter group actually gave rise to flies on the second day, the one fly a male (small), the other a female of medium size. Three flies which issued on the fourth day were large females. Generally speaking the development of small flies is more rapid than that of large ones independent of sex.

Adding the time required for pupal development to the time spent in the larval stage, the insect requires under the conditions stated about 20 to 25 days for its development from the time it issues from the egg to the issuing of the fly from the pupal covering. Naturally other conditions of temperature and natural surroundings will exert a material influence upon the rate of development.

Grassi¹ (1900, p. 84) found that the development of *A. maculipennis* from the time that the larva issued from the egg to the exit of the imago from the pupal case lasted about 30 days at 20—25° C. After about 20 days these flies in turn laid eggs. The development was more rapid in summer, how rapid is not stated. He says (p. 70) that the pupal stage lasts about 3 days. Howard¹ (1900, p. 40) found the pupal stage of the same species (no temperature given) to last not less than 5 days and in several cases as much as 10 days. Finally Ross, Annett and Austen (1900, p. 20) found a species of *Anopheles* in Sierra Leone to undergo pupal metamorphosis (no temperature given) in 48 hours.

The influence of temperature upon the rate of pupal metamorphosis is very evident from our last observation this season. Two full-grown

¹ See Bibliography, p. 75.

larvae, the only two met with, which were caught in the Granta on October 20th became converted into pupae on the 21st. The imago issued from one pupa on the 27th, from the other on the 28th, that is on the 7th and 8th days respectively. The temperature in the Granta about this time measured 8.3° near the surface. The temperature in the laboratory where the insects were subsequently kept varied between 13 and 17° C.

To be continued.

ON THE BACTERIOLOGY OF NORMAL ORGANS¹.

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THE question of the presence or absence of micro-organisms in the normal organs of the body has occupied the attention of numerous observers; yet, despite the fact that its solution is not accompanied by any great difficulties, the results of experimentation have been strangely at variance. For instance, Meissner stated that no bacteria capable of development are present in the living tissues of the healthy animal, while Zweifel² (1885) expressed the opposite opinion, namely, that human organs habitually contain germs, that these germs, however, are anaerobic in character, their capability of development being limited by the amount of oxygen present in the blood stream.

Hauser³ (1885), who took cultures directly from healthy tissues by the ordinary laboratory methods, and later submitted these tissues to microscopic examination, found almost without exception that they were quite free from bacteria. Welch⁴ in 1891, speaking with especial reference to the *Bacillus coli*, stated that he had isolated this organism from the internal organs when some distinct lesion of the intestinal mucosa was present, and almost uniformly failed to find it outside the intestinal tract when no demonstrable lesion of the mucosa existed. He stated moreover that the *Bacillus coli* does not invade the blood and organs in the process of post-mortem decomposition.

¹ The following paper is an abstract of a contribution to the meeting of the Association of American Physicians, held in Washington, May, 1900.

² Zweifel, *Ref. Baumgarten's Jahresbericht*, 1885, Vol. I. p. 168.

³ Hauser, *Ibid.*, 1885, Vol. I. p. 166.

⁴ Welch, *American Journal of Medical Sciences*, 1891, Vol. CI. p. 439.

Neisser¹ (1896) and Opitz² (1898), working along quite independent lines, carried out a number of experiments in relation to this question, and reached almost identical conclusions.

Neisser removed the liver, spleen, kidneys, heart, lungs and mesentery from rabbits and guinea-pigs and placed these organs on nutritive media. He observed the usual precautions to avoid contamination. He found that cultures taken at the end of *two days* were invariably sterile, and concluded therefore that "under normal conditions no bacteria are present in the lymph or blood stream." When on the other hand he first produced artificial lesions of the intestines, and then fed the experimental animals on cultures of various micro-organisms, he found in a considerable portion of cases that he obtained abundant growths from the organs of these animals, either of the bacteria which had been originally administered or of representatives of the normal intestinal flora.

Opitz removed portions of mesenteric glands of cattle killed at the Breslau abattoir. He took them to the Hygienic Institute in that city, where, after carefully sterilising the surface, he excised small pieces and cultivated them on agar and gelatin plates. Finding in the majority of the tissues submitted to this treatment that no bacteria had developed at the end of *three days*, and that the organisms which were isolated from the small number of tissues which decomposed, were in all cases spore-bearing bacilli (including the *Bacillus subtilis*, the spores of which are notoriously resistant), Opitz concluded that the mesenteric glands of cattle are normally sterile, that is, that a passage of bacteria through the intestinal wall during digestion does not normally occur.

Neither Neisser nor Opitz record any observations on organs cultivated for a longer period than three days, and in the experiments of the latter, post-mortem changes cannot be positively eliminated, since a considerable time necessarily elapsed between the death of the animals at the abattoir and the taking of the cultures at the Hygienic Institute. To say the least, Opitz was not dealing with absolutely normal structures.

Recent advances in our knowledge of the etiology of chronic fibroid changes in the organs of man, such changes being produced by the long-continued action of bacteria on the tissues, together with the

¹ Neisser, *Zeitschr. f. Hygiene u. Infektionskr.*, 1896, Vol. xxii. p. 12.

² Opitz, *Ibid.*, 1898, Vol. xxix. p. 505.

special observations of Dr Adami¹ in Montreal on the presence of minute bodies within the cells of normal and diseased livers, which bodies could only be interpreted on the supposition that they were bacterial in nature, has awakened fresh interest in the question under discussion.

As a consequence a series of experiments has been carried out under Dr Adami's direction, with a view to determining if possible whether more positive results were in any way obtainable.

Methods.

A number of animals, rabbits, guinea-pigs, dogs and cats, in conditions of fasting and of full digestion, were killed instantaneously by a blow on the back of the neck or by the use of large quantities of chloroform. Immediately after death the skin was removed from the abdomen and a linear incision made through the abdominal wall. The instruments for this purpose were sterilised by boiling and immediately before use were thoroughly heated in the free flame of the Bunsen burner. With fresh instruments, sterilised in the same way, the two kidneys and portions of the liver were excised, and after being cooked in the flame until the surface had been thoroughly scorched to a greyish-brown, they were at once transferred to culture-media. Three varieties of media were employed. Wide-mouthed bottles containing 50 c.c. of melted gelatin, provided with ground-glass stoppers perforated by small openings plugged with cotton, through which cultures could be taken—served for those organs which were to be preserved at room-temperature. Petri dishes, two inches in depth and two inches in diameter, half filled with neutral agar, were utilised for the cultivation of organs at the body-temperature. The covers of these Petri dishes were loose and could be lifted from the lower portion sufficiently to allow a culture to be taken from the enclosed organ, without exposing the surface of the agar to the chance of contamination from the air. Finally, large test-tubes containing 50 c.c. of neutral broth and provided with inverted inner tubes similar to those used in the Durham modification of the Smith fermentation-tubes, glass rods being passed through the cotton plugs, were employed for "control cultures," corresponding portions of liver and kidney being thus preserved both

¹ Adami, *British Med. Journ.*, Oct. 22, 1898. *Journ. American Medical Association*, Dec. 23, 1899.

on agar and in broth. Broth tubes were used to determine whether a greater variety of bacteria would develop in fluid than in solid media.

All media were sterilised in the autoclave and at the moment of operation the hot sizzling organs were passed from the flame directly into the agar plates, gelatin bottles or broth tubes, the organs in the latter being thoroughly broken up by the glass rod which was withdrawn through the cotton plug. The organs were then incubated at various temperatures. Cultures were made at the end of the first and third days, by drawing up some of the juice of the organ in a sterile glass pipette, subsequent cultures being made every third day till positive results were obtained or till a sufficient time had elapsed, to demonstrate that the organs were absolutely sterile.

As a later modification of this method, the organs after excision, instead of being passed through the flame, were soaked for an hour in a 1—1000 solution of bichloride of mercury, after which they were washed in sterile water and preserved on sterile plates, either dry, or covered with neutral agar. By this means an insoluble precipitate of albuminate of mercury was formed on the surface of the organ, such a precipitate allowing a complete sterilisation of the surface, without permitting the passage of the sublimate into the interior of the organ and the consequent destruction of any bacteria there present.

*Results*¹.

From the organs of *rabbits* preserved in gelatin at a temperature of 22° C., bacteria were grown in 8 out of 12 cases. These cultures were obtained from the 4th to the 17th day. The organisms isolated were *Staphylococcus albus*, *B. coli communis*, *B. proteus*, and several varieties of pathogenic spore-bearing bacilli, the properties of which have been described in another paper.

A number of organs preserved in this way failed to show the presence of bacteria at any time, although cultures were taken at intervals for nearly six weeks.

The cultivation of the rabbit's organs in agar gave results which differed considerably from those obtained with the employment of gelatin. Whatever bacteria were present could develop more rapidly at a temperature of 37° C., and by the end of the fifth or sixth day

¹ For full tables of the results of these experiments see: *Trans. of the Assoc. of American Physicians*, Vol. xv.

many of the organs showed evidences of decomposition, an abundant growth of micro-organisms frequently appearing on the surface of the agar, the bacteria evidently making their way from the enclosed organ through the medium to this point. Cultures were taken from this growth on the agar whenever it appeared, the organ was then removed from the Petri plate, its surface seared with a hot scalpel, and another culture taken from the interior.

The bacteria isolated from the organs preserved in agar were, the *Staphylococcus aureus* and *albus*, *B. mesentericus vulgatus* and *fuscus*, *B. proteus*, *B. paracolon* and some pathogenic spore-bearing bacilli.

The corresponding cultures of the same organs, placed in the broth fermentation-tubes, were identical with the cultures obtained from agar.

In all cases the species of bacteria observed on the surface of the agar plates, were later isolated from the interior of the organ. At the same time, whatever bacteria were obtained from the organs preserved in agar, were grown from the broth tubes as well. The converse of this was not true, however, as in several instances no bacteria were observed in the agar plates while an abundant growth was obtained in the Smith tubes. Evidently the thorough disintegration of the tissues in the fluid medium was more favourable for the development of bacteria, than the preservation of the unbroken organ on a solid substance like agar or gelatin.

Thirty different organs were removed from rabbits and studied in gelatin, agar and broth. Twenty (66%) of these organs contained bacteria. The positive results were obtained from the 4th to the 6th day on agar; from the 4th to the 17th day on gelatin; *cultures taken on first and third days were sterile*.

From the organs of *guinea-pigs*, 18 in number, cultures were grown in 11 cases (61%). The organisms were identical with those seen in the organs of rabbits, and included *Staphylococcus albus* and *aureus*, *B. mesentericus*, *B. proteus*, *B. subtilis* and *B. coli communis*; they appeared in the cultures taken on the *seventh day*.

The organs of *cats* furnished most interesting contrasts with the results obtained on the other species of animals. Bacteria were observed in 77.7% of the samples, 18 organs being studied. These consisted of *B. mesentericus*, *B. proteus* and *B. coli*, associated with *B. megatherium* and *B. mycoides*.

Besides these, two varieties of bacilli with spores, one a long narrow bacillus, the other a short thick bacillus, were observed in the broth-

cultures and in smears made directly from the juice of the organs. These bacilli did not grow on any of the usual media although repeated attempts to effect their cultivation were made both aerobically and anaerobically.

In dogs, apparently the same two uncultivable organisms were observed accompanied by *B. megatherium*, *B. mycoides*, and rarely by *B. zopfii*. Of the 18 dogs' organs examined 15 (83·3 %) contained bacteria. With both dogs and cats the positive results were obtained on the *seventh day*.

The sterilisation of the surface of the organs by bichloride of mercury, with which 12 organs from rabbits were treated, did not influence the results to any material extent. Thus nine (75 %) of these organs decomposed, the bacteria isolated from them being identical with those obtained with the first set of rabbits: *Staphylococcus pyogenes albus*, *B. mesentericus* and *B. coli*.

The microscopic examination of sections of the organs revealed in many cases well-formed bacilli similar morphologically to those obtained on the culture-media. In other cases only a profusion of coccoid and diplococcoid bodies could be seen, a greater abundance of these bodies being found in the decomposing than in the sterile organs.

The number of these bodies was greater in organs from which *B. coli* was grown, than in those from which spore-bearing bacilli like *B. mesentericus* or *B. subtilis* developed.

In the experiments here described, 34 animals were killed, 96 different organs excised and cultivated, and 69 found to contain bacteria. That is, less than 30 % of the organs were sterile. Such results are in more or less direct contradiction to those recorded by other observers, and it thus becomes of interest to seek for some underlying principle which may explain this difference.

The first suspicion to arise in considering our observations, is, that the positive results were due to *contamination* of the culture-media employed, and that greater attention to the details of asepsis might have insured a larger proportion of negative results.

Putting aside, for the moment, the fact that in every experiment the greatest possible care was taken in the proper sterilisation of media and instruments before operation, while at the operation itself the organs were removed as aseptically as possible; putting aside moreover the fact that control cultures were made in every instance, and that

these controls remained free from bacteria, considerable interest of a general character attaches itself to the results obtained.

(1) *Each species of animal* showed its peculiar bacteriology, regardless of the conditions under which the animals were killed or the method in which cultures of the organs were made.

Thus *Staphylococcus albus* and *aureus*, *B. mesentericus*, *B. proteus* and certain pathogenic spore-bearing bacilli were found in the organs of rabbits and guinea-pigs, while *B. mycoides*, *B. megatherium* and *B. zopfii* were found in those of dogs and cats, together with two varieties of undetermined bacteria already alluded to.

(2) *Each animal*, regardless of its species, possessed its distinctive bacteriology. For instance, the same organism was frequently obtained in all the different preparations made from any one particular animal, while in other cases, the entire set of cultures remained sterile.

(3) *Each organ* preserved its own bacteriology. That is, when any bacteria were isolated from the portions of organs kept in agar, they could always be detected in those portions in the Smith tubes, often, however, associated with other bacteria. Similarly the bacteria found in the growth on the surface of agar were always present in the mixed cultures from the organs.

In short, the cultivation of different bacteria from different species of animals, from different animals and from different organs, regardless of the means employed in the sterilisation of the surface of these organs and regardless of the method of their preservation, demonstrates that these bacteria were present in the organs at the moment of death and were not introduced from without by careless manipulation of instruments or media.

When we compare the methods employed by us with those of others, for instance, Neisser and Opitz, we are struck by the difference in *time* which was allowed to elapse between the preliminary excision of the organs and the final verdict as to their bacterial condition. With the former observers, all records were taken at the end of *two* and *three* days, when the organs were described as sterile. In this work the cultures taken at this time were likewise sterile, thus confirming Neisser and Opitz' results. As these organs were preserved for *longer* periods however, positive cultures of bacteria were grown in a large proportion of cases, usually on the *6th*, *7th* or *8th* days, but in some instances as late as the *17th* day. We are thus dealing with a *delayed growth* of bacteria which is somewhat difficult to explain. Either the bacteria are present in small numbers in the organs

at the moment of death, and only develop gradually after the organs are excised, or the normal bactericidal or inhibitory substances of the organs, originally powerful enough to prevent the growth of the bacteria are gradually decomposed after the connections between the organs and the animal body are broken, thus permitting of a slow development on the part of the micro-organisms.

In whatever manner these latter questions may be answered, I must maintain, as a result of the experiments here cited, that at least 70 % of the internal organs of our ordinary domesticated animals contain bacteria which are capable of development provided a sufficient time be allowed to elapse between the removal of the organs and their final examination.

I wish to express my thanks to Dr Adami for his constant advice and aid in the conduct of these experiments.

IN MEMORIAM.

WALTER MYERS.

ANOTHER name has been added to the death-roll of brave workers who for the love of science and the welfare of mankind have gone forth to investigate a most fatal disease.

Walter Myers was born in Birmingham, where he received his preliminary education. In the year 1888 he entered London University, and in 1890 the University of Cambridge. In 1892 he proceeded to the degree of B.Sc. (London) and of B.A. (Cambridge), and two years later entered upon his medical studies at St Thomas's Hospital. In 1897 he qualified as M.B., B.C. (Cambridge), M.R.C.S., and received the degree of M.A. at Cambridge. From October 1897 to October 1898 he worked under the late Professor Kanthack in the Pathological Laboratory of the University of Cambridge, after which he studied under Professor Ziegler at Freiburg, and more especially under Professor Ehrlich in Berlin, and subsequently in Frankfurt. The direction he took in his researches was greatly influenced by Kanthack and Ehrlich.

The experimental researches of Walter Myers relate chiefly to the action of snake-venoms. In his first paper, which was published in conjunction with Dr Stephens, experiments are reported upon the action of cobra venom on the blood, both *in vitro* and *in corpore*, and also upon the neutralising effects of antivenomous serum. It was found that the haemolytic effect of venom was arrested by the serum, the interaction of toxin and antitoxin obeying the law of multiples. The action of the serum proved to be specific. It was also found that larger doses of venom which had been rendered non-haemolytic (through mixture with antivenomous serum) nevertheless proved lethal to experimental animals.

In a paper of which a preliminary report appeared in April 1898, Myers showed that an emulsion made from the cortex of the fresh suprarenal glands of the sheep, bullock, rabbit, and guinea-pig, and also suprarenal tabloids, checked the lethal effect of small quantities of

cobra venom, and that this property was confined to this organ. The medulla of the suprarenal gland had no such effect, and it is owing to the glands in the guinea-pig being chiefly composed of medulla that the suprarenals of this animal exerted but a slight influence. In view of the lethal effect of multiples of the minimal lethal dose of venom injected together with suprarenal emulsion, Myers concluded that the latter acted only by raising the natural resistance of the body.

In May of the same year Myers published a paper on the standardisation of antivenomous serum, in which he criticises the methods hitherto employed, and describes a cheaper and more accurate method whereby the minimal lethal dose can be determined to within 20% by the use of mice as test animals. A paper on the interaction of toxin and antitoxin, illustrated by the reaction between cobra-lysin and its antitoxin, followed next, and is the longest paper he wrote. Then followed a communication in which he discusses the theories as to the cause of the shape of non-nucleated red corpuscles.

Perhaps the most important paper published was the last one, which appeared shortly after his return from Frankfurt as a preliminary note, entitled "Immunity against Proteids." Myers injected solutions of crystallised egg-albumen, sheep's and bullock's serum-globulin, and Witte's peptone into different rabbits, and after a time noted the appearance of specific precipitins in their serum. His results strongly support the view that the production of immunity is due to a process of assimilation.

Whilst engaged upon these researches in Cambridge he was invited to become a member of the Yellow Fever Expedition of the Liverpool School of Tropical Medicine. He left England in June 1900 in company with his Cambridge colleague Dr Durham for America. They journeyed through Canada and the United States, and proceeded thence to Cuba, where they met the United States Yellow Fever Commission, which was engaged in research at Havana. In August 1900 they reached Pará, Brazil. On the 16th of December both investigators were attacked by yellow fever within a few hours of each other, the last act of Walter Myers having been to take his colleague to the hospital where he himself was soon to succumb from a malignant form of the disease. Dr Durham most fortunately recovered after a mild attack. A preliminary report of the results of the expedition has recently appeared in the *British Medical Journal* (23 February, 1901).

The medical profession of this and other countries has sustained a severe loss in being deprived of so promising a member, whilst the loss



WALTER MYERS

Born at Birmingham, 28 March, 1872.

Died at Pará, Brazil, 20 January, 1901.

to his family is irreparable, for he was an only son. He was cut off at the beginning of a career for which he was eminently fitted both by natural endowment and thorough special training. The work which he accomplished in the brief period which was allowed him will endure.

Though he was associated but for a brief period in the work of the Liverpool School of Tropical Medicine, it is a source of gratification to his friends to learn that his memory will be perpetuated in that Institution through the foundation of a "Walter Myers" Chair and Scholarship in Tropical Medicine.

The accompanying portrait was taken in the Pathological Laboratory at Cambridge shortly before his departure. We are indebted to Dr Louis Cobbett for kindly placing the negative thereof at our disposal.

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G. H. F. N.

MAXIMILIAN VON PETTENKOFER.

Whilst the *Journal* is going to press we receive the news of Professor von Pettenkofer's death, which took place in Munich on the 10th of February at the age of 83 years. A biography and portrait will appear in our next number.

G. H. F. N.



MAX VON PETTENKOFER

Born at Lichtenheim, 3 December, 1818.

Died at Munich, 10 February, 1901.

THE WORK OF MAX VON PETTENKOFER.

THE death of Professor von Pettenkofer, which was briefly alluded to in the last issue of the *Journal of Hygiene*, has removed one who was everywhere acknowledged as a great leader among the workers in our science, and as the pioneer who has been mainly instrumental in bringing about the recognition of Hygiene, particularly on its experimental side, as a separate subject of investigation and university instruction.

Pettenkofer was born in 1818 near Neuburg in Bavaria. His parents were very poor, but when he was nine years old an uncle, who was druggist to the Court at Munich, undertook to provide for his education. After finishing his course at the "Gymnasium" he studied for two years at the University of Munich, and when 21 years old was apprenticed as a druggist. Not content with this work he suddenly gave it up, and became for a short time an actor, but after a few months returned to Munich and worked for a degree in medicine, which he obtained in 1843.

He had already become interested in scientific investigations, and now determined not to practise, but to obtain if possible a university position in which he could devote himself to science. He went accordingly to work in Scherer's laboratory at Würzburg, and later to the still more famous laboratory of Liebig at Giessen. The subject to which he specially devoted himself during the year thus spent, was Physiological Chemistry; and he very quickly made for himself a name by his discovery of creatinin in urine, and of the familiar test for bile acids which is so well known as "Pettenkofer's reaction."

On his return to Munich the University authorities recommended to the Government that a Professorship of Physiological Chemistry should be founded and conferred on Pettenkofer. This recommendation was however set aside, and he had to content himself with a subordinate post as chemist at the Mint. While at the Mint he carried out important investigations on the chemistry of metals, adding considerably

to his reputation: at last, in 1847 on a change of ministry, he was appointed to a special Chair of Pathological Chemistry. In 1850 he presented to the Bavarian Academy a remarkable paper, in which he drew attention to some of the main facts which form the basis of what is now so well known as the Periodic Law of the Elements. The importance of this paper was not recognised by the Academy, which, to Pettenkofer's great disappointment, declined to give him the means of carrying on the investigation. Pettenkofer thus shared the fate of Newlands in this country. Nearly fifty years later (in 1899) the German Chemical Society conferred on him the Liebig medal in tardy recognition of the merits of his work of 1850.

From about 1850 onwards Pettenkofer directed his attention more and more to investigations in Hygiene, and in 1853 he began to give courses of lectures devoted entirely to the subject. A Chair of Hygiene was founded for him in 1865, and in 1878 the famous laboratory of Hygiene at Munich was built under his direction. He held the Munich Chair until 1894.

The *Zeitschrift für Biologie*, which was devoted partly to Physiology and partly to Hygiene, was founded by Pettenkofer, Voit, and two of their colleagues in 1865. In it appeared until 1883 nearly the whole of the important series of investigations carried out by Pettenkofer and those associated with him. In consequence of the increasing importance of the subject of Hygiene, he, in conjunction with Professors Forster and Hofmann, founded the *Archiv für Hygiene*, of which he continued to be one of the editors until 1895.

It is only possible here to give a very short account of Pettenkofer's scientific work in connection with Hygiene. His published papers deal mainly with three lines of investigation, relating respectively to nutrition, to certain epidemic diseases, and to vitiation of air.

In his work on nutrition and dietetics Pettenkofer was associated from the beginning with his colleague Professor Voit. The first step in their investigations was the invention by Pettenkofer of his famous respiration apparatus, which was erected with the help of a grant of £1000 from the King of Bavaria, and described in 1858. Pettenkofer's object in constructing it was to obtain complete information as to all the sources of gain and loss to the body over a given period, the man or animal experimented on being kept under perfectly normal conditions. In previous experiments on nutrition and the effects of various diets the nitrogen, but not the carbon, oxygen and hydrogen leaving and entering the body had been determined, so that there

was then only an imperfect knowledge of the relation of diet to the oxidation of material in the body. How incomplete such knowledge was will be evident from our example. We now know, thanks to the use of the respiration apparatus, that during starvation, although the excretion of nitrogen, which is an index of proteid oxidation, may fall to a fraction of its normal value, yet the total amount of material oxidised remains about the same; for although the oxidation of proteid is diminished, this diminution is compensated for by increased oxidation of fat. Proteid, carbohydrate and fat are in fact capable, within certain limits, of replacing one another; and the proportion of one of these substances which replaces another depends, as shown by Rubner, on the relative energy-values of the two substances. When, for instance, fat is substituted for proteid, the amount of energy liberated in the body by the oxidation of the proteid replaced is equivalent to that liberated by the fat substituted for it. Observations merely on proteid metabolism, as indicated by the nitrogen of the urine, give, therefore, only very partial, and by itself misleading, information as to the consumption of material in the body.

With characteristic insight Pettenkofer perceived the necessity of observing over considerable periods not only the excretion of nitrogen and other constituents of the urine and faeces, but also the output of carbonic acid and intake of oxygen. The respiration apparatus, which was further improved by Voit, furnished the means of obtaining the latter data, and thus rendered possible the long and very important series of investigations carried out at Munich by Voit, Pettenkofer and their pupils. Through these investigations a far more complete insight has been obtained into the part played in nutrition by the different constituents of food, the effects of variations in the quantity of food taken, and many other important questions. It may be said with strict truth that the experiments of the Munich school form the chief basis of our present knowledge of dietetics.

The second line of investigation pursued by Pettenkofer relates to the causes of epidemic disease, in particular cholera and typhoid. About the time when he settled in Munich both cholera and typhoid were formidable sources of danger, and naturally attracted his special attention. In Munich the annual death-rate from typhoid alone was sometimes as high as 3 per 1000, or about 15 times the present rate in England. Pettenkofer set himself to investigate the causes of the prevalence of these two diseases; and he continued his investigations over forty years. The conclusion to which he was led, and which he

continued to the end to urge with undiminished vigour and ability, was that although cholera and typhoid are due to specific organisms, introduced from without, these diseases are nevertheless not spread as epidemics by immediate infection from person to person, as in the case of small-pox and many other infectious diseases. For their epidemic spread certain local conditions are necessary; and apart from the presence of these conditions the mere introduction of the organism causing typhoid or cholera is of little importance. In support of this view he pointed both to experience of the slight danger from direct contact with patients, and to the fact that many places remain immune to cholera or typhoid although the germs of the disease are constantly being introduced by infected persons coming from elsewhere. As the result of long investigations at Munich and elsewhere, he inferred that the chief local condition for the spread of cholera and typhoid is a soil charged with organic impurities and in a certain condition of dryness and porosity. With regard to typhoid in particular he showed that its prevalence in Munich coincided with a fall in the level of the ground-water, which implies drying of the soil. He further maintained that such measures as quarantine, disinfection, and isolation are ineffective in practice against cholera and typhoid, whereas by keeping the soil pure by an effective system of sewers, with sufficient water-supply to carry away all impurities, epidemics of cholera and typhoid may be effectually prevented. He vigorously opposed the theory that typhoid and cholera epidemics are caused by the drinking of water contaminated directly by the excreta of persons suffering from these diseases; and both he and his pupil Emmerich performed on themselves the daring experiment of swallowing a fresh culture of the comma bacillus, after neutralising with soda the contents of the stomach. In each case the result was a watery diarrhoea, the discharge being an almost pure cultivation of the comma bacillus. The accompanying symptoms were, however, very slight as compared with typical cholera, and Pettenkofer considered, whether rightly or wrongly, that the experiment confirmed his views.

On going through the great mass of evidence contained in Pettenkofer's writings, and particularly the facts with regard to Munich, where typhoid, in consequence apparently of the purification of the soil by a proper drainage system, has now almost disappeared, it is difficult to come to any other conclusion than that his contentions embody a very important element of truth. On the other hand the proof of the spread of cholera and typhoid in particular epidemics by water is now so strong

that few will follow him in denying that this means of spread is of common occurrence.

Pettenkofer's third main line of investigation was connected with the principles of ventilation, and the manner in which the air of inhabited buildings becomes vitiated. His earliest published work on this subject contains a description of his well-known method for the determination of CO_2 in air. A very similar method had, unknown to him, been used by Dalton and Watson in England; but it was Pettenkofer who gave the method its present form, and at the same time showed how it could be made use of for measuring the degree of vitiation of the air of rooms. With the help of this method he and his pupils investigated the proportion of CO_2 which corresponds to a reasonable standard of purity in the air of inhabited rooms, the rate at which air passes through the walls of a closed room under various conditions, the composition of ground air and its penetration into houses, the vitiation of air by lights, the efficiency of various systems of ventilation, and other important questions. A further series of experiments carried out in his laboratory related to carbonic oxide poisoning from the passage of lighting gas through the soil into houses from broken gas mains, the deodorisation of this gas in its passage through the soil, and the effects on men and animals of small proportions of CO in the air breathed.

It is not merely for the importance of the actual scientific work which he accomplished that Pettenkofer will be remembered: for it is chiefly through his influence that Hygiene has secured recognition as a definite branch of applied science, based solely on careful and accurate observation and experiment. The great importance to the community of practical measures relating to Public Health has for long been recognised and acted upon, particularly by statesmen and leaders of medical opinion in England. Pettenkofer not only helped to secure this recognition in Germany, but insisted on the equal importance of obtaining a sound scientific basis for all practical measures carried out by the community in the interests of Public Health. Largely in consequence of his efforts Germany has now chairs of Hygiene with efficiently equipped laboratories in all but one of her twenty universities, besides the celebrated Kaiserliche Gesundheitsamt at Berlin, of which the directorship was at first offered to him. It is only necessary to consider the work already accomplished in connection with these laboratories in order to see how great has been the benefit to humanity which their establishment has conferred. England, which was formerly far ahead in matters of Public Health, has unfortunately

lagged behind in recent years. The scanty and inadequate provision made in this country for the advance of that definite knowledge which is the only sound basis for administrative work in Hygiene contrasts very unfavourably with what now exists in Germany.

Much of Pettenkofer's influence was due to his genial personality, and to the power, which he possessed in an extraordinary degree, of clearly and vigorously expressing his thought, and carrying with him the interest of his readers. Of all his numerous papers not one is dull or obscure.

He resigned his chair in 1894, and the editorship of the *Archiv für Hygiene* shortly afterwards. He felt himself no longer physically fit for active work, although, to judge from his latest writings, he was mentally as vigorous as ever. A pathetic interest attaches to what he wrote at the beginning of his description of the experiment—one of the last he performed—in which he swallowed a cultivation of the cholera-bacillus.

"On the 7th of October, 1892, in the presence of witnesses, I took this cholera-drink, which tasted like the purest water. Some of my friends were concerned for me, and asked that if I were determined that the experiment should be made they might be allowed to sacrifice themselves in place of their old teacher; but I wished to act according to the old maxim: *fiat experimentum in corpore vili*. I have the right to consider myself as a *corpus vile*. I am 74 years old, I have suffered for years from glycosuria, have not a single tooth left, do not even use my artificial teeth in eating, but only when I have to speak long and clearly, and I feel also other burdens of old age. Even if I were mistaken and the experiment endangered my life I should look death calmly in the face, for it would be no thoughtless and cowardly suicide: I should die in the service of Science as a soldier on the field of battle. Health and life, as I have often said, are very high earthly gifts, but not the highest for man. The man who wills to stand higher than an animal must be ready to sacrifice even life and health for a higher ideal good."

On retiring from active work he went to live at his house in the country, and those who visited him there found his welcome as genial, and his interests as keen as ever, although he evidently felt deeply his separation from the work he loved so well. The news of his sudden end while in a fit of deep depression came as a shock to the many, in all civilised lands, who honoured him not more for the greatness of his work than for the greatness of his character.

THE RELATIVE ABUNDANCE OF BACILLUS COLI COMMUNIS IN RIVER WATER AS AN INDEX OF THE SELF-PURIFICATION OF STREAMS.

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It is well known that fresh sewage always contains large numbers of the common colon bacillus. It is also true that when a water source is polluted with any considerable quantity of fresh sewage it is usually possible to demonstrate the presence of the colon bacillus in such water. Upon these familiar facts have been based various methods and conclusions of greater or less value to public hygiene.

The particular method of gauging the so-called self-purification of a stream by the relative abundance of *B. coli communis* at different points is not a new one, but, so far as the writer is aware, it has not been often applied on a large scale.

Theobald Smith's ingenious method of estimating the approximate number of faecal bacteria in water by the fermentation tube was first used in the study of the self-purification problem by Smith and Brown⁽⁸⁾. The work of these authors was carried out upon the Mohawk and Hudson Rivers under the auspices of the New York State Board of Health, and aided materially in the solution of local problems. Owing, however, to the relatively low degree of pollution obtaining in these particular river waters, as is shown both by the chlorine determinations and by the small number of faecal bacteria, the contrast between different points along the course of the river is not very marked.

Some investigations also have been carried out by Hammerl⁽⁴⁾ upon the river Mur above and below Gratz, but the number of this author's determinations, as recorded in his article, are so few, and his method¹

¹ Suspicious colonies upon a gelatin plate were fished and tested "for ability to grow at blood temperature, to curdle milk and to produce gas in media containing sugar," *op. cit.*, p. 537.

for the detection and enumeration of colon bacilli is so inadequate that not much weight can be attached to his conclusions.

From a general standpoint it is clear that the question of self-purification can be most advantageously studied where the proportion



of sewage added to river water is very high, so that slight fluctuations due to temporary and local conditions, to incomplete mingling and to other minor factors, are wholly submerged by a gross and constant

pollution. A particularly favourable opportunity has been recently afforded the writer in the course of an investigation of the conditions created in the Illinois Valley by the discharge of the sewage of the city of Chicago into the Desplaines River. This stream unites with the Kankakee River, nineteen miles below the point where the Chicago sewage is received, to form the Illinois, a stream flowing 268 miles to the Mississippi (see map, p. 296). The enormous initial pollution and the fact that during certain seasons of the year the dilution from rainfall and run-off is slight¹ render it a comparatively easy task to trace the progressive purification in the flowing stream. Few other rivers, in which the process of self-purification has been studied, present so fortunate a union of three important conditions: extreme pollution, relatively little dilution, and great length.

During the studies of the Illinois River upon which the writer has been recently engaged in behalf of the Sanitary District of Chicago he has had occasion to determine the relative number of faecal bacteria in the river water at different points. The methods employed and the results obtained are presented here through the courtesy of Dr Arthur R. Reynolds, Director of the Streams Examination².

¹ A chlorine content as high as 40 (parts per million) has been found at the mouth of the Illinois.

² For the sake of clearness the local conditions leading to the inquiry may be briefly summarized. (For a fuller description see (6).) A large part of the sewage of the city of Chicago has for many years flowed into the Chicago River, which being of small volume has become practically an open sewer. To prevent pollution of the water supply by the discharge of this sewage into Lake Michigan a pumping-station at the union of the South Branch of the river with the Illinois and Michigan Canal (Bridgeport, see map) has been utilized for pumping the river water into the canal. The amount so discharged has been estimated at about 35,000 cubic feet per minute. The canal unites with the Desplaines River a little distance above Joliet (see map), and by this route a large part of the sewage of Chicago has made its way down the Illinois Valley. As is seen by the map the canal is continued to La Salle, from which point the Illinois River is navigable to its mouth. As might be supposed, however, comparatively little sewage passes by this circuitous route. The large and expensive Sanitary Canal, which has been just completed, is designed to carry off the sewage by gravity flow, at the same time greatly diluting it with the purer water of Lake Michigan, and providing for the disposal of the sewage of the whole district. By this means the current in the Chicago River is permanently reversed, and all danger of pollution of the water supply from this source effectually prevented. The Sanitary Canal (not shown on map) runs nearly parallel to the old Illinois and Michigan Canal as far as Lockport, and discharges into the Desplaines River bed at this point. The flow prescribed by law is 300,000 cubic feet per minute for the present population of Chicago. The controlling gates of the Sanitary Canal at Lockport were first opened on January 17th, 1900.

Methods.

In the beginning of the work use was made of the method of direct inoculation of water into the fermentation tube as suggested by Smith in 1893, but this procedure was soon abandoned in favour of another method which was continued through the major part of the investigation. This consisted in a preliminary incubation of a measured quantity of water in carbol-broth. The carbol-broth was prepared by adding 1 c.c. of a 1% solution of carbolic acid in sterile water to tubes containing 9 c.c. of sterile broth of the standard composition. The use of measured quantities of fluid in this way of course necessitates allowance for evaporation during sterilization. By careful attention to the size of the tube and to the period of sterilization in the Arnold steam sterilizer, it has been found possible to calculate very closely the loss of fluid during heating and to make due allowance for it; subsequent evaporation before use has been guarded against. The carbol-broth which we have used has been first rendered neutral to phenolphthalein and then acidified by the addition of 5.5 c.c. of normal acid per litre.

In carrying out the method, 1 c.c. of a suitable dilution of the water has been added to a tube of carbol-broth and incubated at 38° C. for 18—24 hours. Platings from this broth have then been made in litmus-lactose-agar (5 c.c. normal alkali per litre). If red colonies developed on the medium at 38° C. they were transferred to tubes and tested at once for (1) Gas-production in dextrose-broth in the fermentation tube; (2) Indol-production in sugar-free broth; (3) Coagulation of milk; (4) Liquefaction of gelatin.

During a part of the investigation another method was employed consisting of the introduction of water directly into dextrose-broth fermentation tubes without preliminary incubation. The dextrose-broth was prepared with fresh meat from which the muscle-sugar had been removed by Smith's method, and to this sugar-free broth 1% of dextrose was subsequently added. The broth was made neutral to phenolphthalein. After inoculation with the water the tubes were incubated at 38° C. for 48 hours, gas readings being taken at 24-hour intervals. At the end of 48 hours all tubes showing the formation of gas were removed from the incubator, cooled to the room temperature and the absorption of CO₂ determined by the addition of a 2% solution of NaOH. It has been found necessary to take precautions against

incomplete absorption of the CO_2 , especially where large tubes are used and the amount of gas formed is considerable.

The use of litmus-lactose-agar for plating water direct proved entirely unadapted to the conditions of our work, and frequently failed to reveal the presence of the colon bacillus when the two methods above mentioned showed conclusively that this bacillus was present.

Although many other methods were experimented with and carefully compared during the course of the work, only findings obtained by the carbol-broth and dextrose fermentation methods are included in the following tables. A comparison of the two methods⁽⁵⁾ showed that while in the main the results tally closely, the carbol-broth method is in general to be preferred for highly polluted waters, while for relatively pure waters the use of the fermentation tube direct appears to be a slightly more delicate test.

The interpretation of the results obtained by the respective methods demands some explanation, since the whole inquiry hinges upon the meaning of the records. It may be said at the outset that no attempt is made in this paper to record separately the occurrence of various members of the colon group of organisms, or to pass judgement upon the sanitary significance attaching to the presence of various kinds of colon and paracolon bacilli. For reasons easily understood such a subdivision of material would be entirely foreign to the immediate problem. A general and arbitrary standard has of necessity been chosen. In the present state of uncertainty among bacteriologists regarding classification within the colon group, I have thought it best for the purposes of this investigation to adopt a somewhat comprehensive grouping and to include among "colon bacilli" (or "faecal bacteria") certain colon-like organisms showing a fundamental biological relationship. It should perhaps be expressly stated that the term "colon bacillus" is employed in this paper in this general sense and is not used to designate a sharply defined single "species."

The carbol-broth method, as described above, has given results variously designated in the tables as +, - or ?. The sign +, as here used, indicates that colonies have been isolated which gave the typical characters of *B. coli* in (1) Fermentation tube (dextrose-broth); (2) Sugar-free broth for indol; (3) Milk; (4) Gelatin.

The sign - is used to denote those cases where, upon plating in litmus-lactose-agar, after incubation in the carbol-broth, careful search failed to reveal any red colonies; under this head also are placed those cases, not very numerous, where pure cultures obtained from a red

colony have failed to yield an excess of H in the fermentation tube¹.

In the doubtful class are included those instances sometimes encountered, where the organism isolated from a red colony produces the typical mixture of gases in the fermentation tube, but fails to respond positively to one or two of the other characteristic biological tests, (2) being the determination most frequently at variance.

The results obtained by the dextrose-broth method are tabulated on the following basis: The sign + is used for those inoculations yielding a total gas production of more than 20% of the tube length and showing on absorption an appreciable excess of H. If pure cultures are isolated from such tubes organisms possessed of the biological characters above cited will almost invariably be found. There is invariably a mixture of different kinds of organisms in these tubes, and it is sometimes necessary to examine a great many colonies. When this has been done we have never failed to find the colon bacillus. The error involved in the assumption that the colon bacillus is always present in these cases is probably small. The close agreement of the results obtained by this method with those reached by the carbol-broth method, which appears on the face to be more rigorous, lends further countenance to this view.

Under the sign — are included those determinations in which no gas or only a small amount of gas—less than 10%—was produced. There is perhaps a larger measure of uncertainty regarding the determinations classified under this head; it is possible that the colon bacillus was present in some instances where no gas or only a slight amount was formed, but these cases must have been rare since we have never been able to isolate the colon bacillus on gelatin or litmus-lactose-agar plates made from such tubes. An exception must of course be made to this statement in cases where sewage or highly polluted water has been inoculated into the fermentation tube without proper dilution, since in such cases it sometimes occurs that only a small amount of gas collects in 48 hours, the colon bacillus being apparently overgrown by other sewage bacteria.

In the doubtful class are placed those determinations giving a total gas production of 10—20%, and those with a total gas production of more than 20% and absorption-test showing an appreciable excess of

¹ The current assumption that the gas remaining after absorption with caustic potash is H is here followed, although it is probable that other gases, such as N and CH₄, are present in small quantities.

CO₂. The majority of the determinations so classified might fairly be regarded as negative and I believe that only a slight error would be incurred if this were done. I have preferred, however, to adopt the more unequivocal arrangement.

The samples of water examined in this work were collected and shipped to the laboratory in the way I have elsewhere described⁽⁶⁾. The effect of the ice-packing upon the number of colonies appearing in the ordinary plate count has been already discussed⁽⁷⁾. There is need, however, to consider briefly in this place the influence of the low temperature upon the number of colon bacilli. Freudenreich⁽²⁾ has called attention to the fact that in both sterilized and unsterilized waters maintained at room temperature after inoculation with *B. coli* there is sometimes an increase and sometimes a decrease. From this he draws the conclusion that if water samples cannot be examined for the colon bacillus immediately after collection they should be packed in ice for transportation to the laboratory. The hasty assumption that no change in the colon content occurs in ice-packed samples obviously needs justification. Our own experiments are too few in number to warrant generalization, but so far as they go they indicate that no material change occurs in ice-packed samples within 48 hours, a period longer than that usually consumed in transportation.

These experiments may be briefly summarized. In five examinations of various natural waters in different dilutions no change was observed in the number of colon bacilli after the water had remained packed in ice for 46—48 hours. In one other examination there was no appreciable change in 24 hours, but a slight decrease was observable after 48 hours. In another examination the colon bacillus was found in 1 c.c. (2 determinations) at the outset, while it was not in 0.1 c.c.; eight days later the proportion was the same: it was present in 1 c.c. (2 tubes), but not in 0.1 c.c. In another ice-packed sample the colon bacillus was found in 0.1 c.c. immediately after collection, and again after the lapse of 24 hours, but not after six days. One typical experiment of this sort may be tabulated here:

	0.1 c.c.	1 c.c.	2 c.c.
Immediately after collection	0 0	+ + + 0	
After being packed in ice for 47 hours, } temp. 1.5° to 3° C.	0 0 0 0	+ + + +	+ + 0 0

Examinations of three separate water samples allowed to stand at room temperature showed neither increase nor decrease after 46—48 hours.

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Taken as a whole our observations clearly indicate that the changes in the colon content of a water which occur on standing are not very rapid and are usually in the direction of a diminution. It may be fairly assumed that the transportation error is less for the colon determinations than for the colony count.

Together with the detailed tables, a few auxiliary data may be presented. I have not been able to discover any statement by authors concerning the number of colon bacilli normally found in fresh sewage and therefore desire to incorporate here a few observations made in my laboratory by Mr W. G. Sackett. Samples of sewage collected from the 56th St. sewer and composed almost entirely of house-sewage were examined immediately after collection with the following results:

*Fresh Sewage*¹.

Date	·00001 c.c.			·0001 c.c.			Chlorine
	+	-	?	+	-	?	(parts per million)
1900							
May 16	2	0	0	—	—	—	—
Nov. 16	0	5	0	—	—	—	66
20	2	2	1	—	—	—	—
21	0	5	0	—	—	—	—
24	—	—	—	3	1	1	70
27	—	—	—	1	3	1	70
30	1	4	0	3	0	2	73
Dec. 4	0	5	0	4	1	0	68
5	3	2	0	4	0	1	62
11	2	3	0	5	0	0	68
12	2	0	3	5	0	0	68
14	3	1	1	5	0	0	66

TABLE I. Illinois and Michigan Canal, Lockport.

Serial number	Date	·00001 c.c.			·0001 c.c.			·001 c.c.			·01 c.c.			·1 c.c.		
	1899	+	-	?	+	-	?	+	-	?	+	-	?	+	-	?
526	Aug. 29										1	0		1	0	
876	Oct. 31				4	0		2	0							
914	Nov. 7	0	4		1	0	1									
950	14	2	2		1	0	1									
982	21	1	1		4	0										
1011	28	0	2		2	1	1									
1042	Dec. 5	0	0	1	1	0	1									
1072	12	0	2		2	1	1									
1110	19	0	2		3	0	1									
1146	28	0	2		4	0										

¹ Collected from the 56th St. sewer between 3 and 4 p.m. and examined within an hour after collection.

Serial number	Date 1900	·0000! c.c.			·0001 c.c.			·001 c.c.			·01 c.c.		·1 c.c.	
		+	-	?	+	-	?	+	-	?	+	-	+	-
1168	Jan. 3	0	2		2	2								
1199	9	0	2		3	1		2	0					
1239	16	1	0		2	0								
1269	23	0	4		4	0								
1303	30	0	4		1	3								
1334	Feb. 6	0	2		2	2								
1457	Mar. 6	0	4		1	3								
1493	13	0	2		2	1	1	1	0					
1522	20				1	2		1	2					
1563	28	1	2		3	0								
1597	April 3	0	3		1	2								
1636	10	1	1		2	0								
1675	17	0	2		0	1	1	1	0					
1717	24	1	0		1	1		0	0	1				
1753	May 2	0	1		0	2		1	0		1	0		
1786	8	0	1		1	0		2	0		1	0		
1817	15	0	1		1	0		1	0					
1856	22	1	1		1	0	1							
1895	29	0	2		1	1								
1923	June 5	0	2		1	0	1							
1959	12	0	2		2	0								
2000	19				0	1		0	1		1	0		1
2032	26				0	2		0	2					

TABLE II. Desplaines River, Lockport.

Serial number	Date 1899	·01 c.c.			·1 c.c.			1 c.c.		
		+	-	?	+	-	?	+	-	?
383	Aug. 1				0	1		0	1	
418	8				0	1		1	0	
498	22				0	1				
527	29				0	1		1	0	
877	Oct. 31				1	0				
915	Nov. 7				0	0	1	0	0	1
951	14				0	1		0	0	1
1012	28	0	3		0	2				

TABLE III. Kankakee River, Wilmington.

Serial number	Date 1899	·1 c.c.			1 c.c.		
		+	-	?	+	-	?
415	Aug. 7	0	1		1	0	
496	22	1	0				
528	29	1	0		1	0	
566	Sept. 4	0	1		1	0	
875	Oct. 30	1	0				
913	Nov. 6	0	0	1	0	1	
981	20				1	0	

TABLE IV. Illinois River, Morris.

Serial number	Date	·0001 c.c.			·001 c.c.			·01 c.c.			·1 c.c.			1 c.c.		
		+	-	?	+	-	?	+	-	?	+	-	?	+	-	?
	1899															
879	Oct. 31				2	1		2	0							
917	Nov. 7				2	1	1	2	0							
962	15	0	2		3	0		3	0	1						
985	21	1	2		2	3		3	0							
1014	28	0	1		4	0		4	0							
	1900															
1196	Jan. 7				1	0	1	3	0	1	2	0				
1209	10				1	0		1	0							
1248	19				1	0		2	0							
1296	27				1	2		2	0		2	0				
1307	30				0	2		2	2		2	0				
1338	Feb. 6							0	4		2	0				
1373	13				0	2		1	3		2	0				
1400	20				0	2		1	2	1	4	0				
1461	Mar. 8				1	1		3	1		2	0				
1500	15				0	1		1	1	1	1	1				
1526	20				0	2		1	0	1	2	0				
1571	29				0	1		1	1		2	0				
1602	April 4				0	1		1	1		0	1				
1640	10				1	0		2	0		1	0				
1680	17				0	1		2	0		1	0				
1715	23				0	1		0	1	1	1	0				
1758	May 2							0	1		0	1	1	1	0	
1784	8							0	1		2	0		1	0	
1822	16							0	1		1	1		1	0	
1854	22							0	1		0	2		1	0	
1893	29							0	1		1	1		2	0	
1928	June 5							1	0		2	0		0	1	
1962	12							0	1		2	0		1	0	
1996	19							0	1		2	0		1	0	
2037	27							0	1		2	0		1	0	

TABLE V. Fox River, Ottawa.

Serial number	Date	·1 c.c.			1 c.c.			5 c.c.		
		+	-	?	+	-	?	+	-	?
	1899									
384	Aug. 1	0	1		0	1				
420	8	0	1		0	1				
500	22	0	1							
529	29	0	1		0	0	1			
881	Oct. 31	0	1		1	0				
918	Nov. 7	0	1		0	1				
953	14	0	1		0	1				
986	21	0	1		0	1				
1015	28	0	1		0	1				

Serial number	Date	1 c.c.			1 c.c.			5 c.c.		
		+	-	?	+	-	?	+	-	?
	1900									
1282	Jan. 26	0	3	1	1	2				
1528	Mar. 21	0	3		0	3				
1568	28				0	2	1	2	0	
1603	April 4	1	0		2	0		1	0	
1642	11				2	0		1	0	
1681	18	0	1		0	2		1	0	
1722	25	0	1		0	1	1	0	0	1
1759	May 2	0	1		0	2		1	0	
1790	9	1	0		2	0		1	0	
1823	16	0	1		0	2		1	0	
1859	23	0	1		0	2		0	1	
1930	June 6	0	1		0	2		0	0	1
1963	12	0	1		0	1	1	1	0	
1997	19	0	1		0	2		1	0	
2035	26	0	1		1	1		1	0	

TABLE VI. Illinois River, Ottawa.

Serial number	Date	1 c.c.			1 c.c.			1 c.c.			5 c.c.		
		+	-	?	+	-	?	+	-	?	+	-	?
	1899												
385	Aug. 1				0	1		0	1				
421	8				0	1		1	0				
501	22				0	1							
530	29				0	1		0	0	1			
880	Oct. 31	0	4		1	0	2						
919	Nov. 7	0	2		1	2	1	1	0	1			
954	14	0	2		2	2		2	0				
987	21	1	1		1	0	3	2	0				
1016	28	0	2		0	3	1	0	0	2			
1076	Dec. 13	1	1		3	1		2	0				
1130	22	1	0		1	0		1	0				
1153	29	1	0	1	1	0	3	0	0	2			
	1900												
1174	Jan. 5	1	0		3	0	1	2	0				
1221	12	0	1		2	0		1	0				
1250	19	0	2		3	0	1	2	0				
1283	26	0	0	2	4	0		1	0				
1340	Feb. 6				1	3		2	0				
1402	21				0	2		3	1				
1455	Mar. 6				0	4		0	19	1			
1490	13	0	2		2	2		2	0				
1529	21	0	1	1	2	0		1	1				
1569	28	0	2		1	1		1	1				
1604	April 4	0	1		0	2		1	0				
1643	11	0	1		0	2		0	1				
1682	18				1	1		0	0	2			

TABLE VI. Illinois River, Ottawa (*continued*).

Serial number	Date	.01 c.c.			.1 c.c.			1 c.c.			5 c.c.		
		+	-	?	+	-	?	+	-	?	+	-	?
1723	April 25				0	2		0	2				
1760	May 2	0	1		0	2		0	0	1			
1791	9				1	0		2	0				
1824	16				1	0		0	0	2	1	0	
1860	23				0	0	1	0	0	2	0	0	1
1931	June 6	0	1		0	1		0	0	2	0	0	1
1964	12	1	0		0	0	1	2	0		1	0	
1998	19	0	1		1	0		1	0		1	0	
2036	26	0	1		0	1		1	1		1	0	

TABLE VII. Big Vermilion River, La Salle.

Serial number	Date	.1 c.c.			1 c.c.		
		+	-	?	+	-	?
	1899						
388	Aug. 2				0	1	
423	9				0	0	1
502	23	0	1		0	0	1
533	30	0	1		0	1	
882	Nov. 1	1	0		1	0	
920	8				0	1	
959	15				0	1	
988	22	0	1		1	0	
1017	28	0	1		1	0	

TABLE VIII. Illinois River, La Salle.

Serial number	Date	.1 c.c.			1 c.c.		
		+	-	?	+	-	?
	1899						
389	Aug. 2				0	0	1
424	9	1	0		0	0	1
503	23	0	1		0	0	1
534	30	0	1		0	0	1
883	Nov. 1	1	0		1	0	
960	15	0	1		1	0	
989	22	0	1		0	0	1
1018	28	0	1		1	0	

TABLE IX. Illinois River, Henry.

Serial number	Date	.01 c.c.			.1 c.c.			1 c.c.		
		+	-	?	+	-	?	+	-	?
107	June 7							0	1	
134	14							0	1	
173	21				0	1		0	1	

Serial number	Date	.01 c.c.			.1 c.c.			1 c.c.		
		+	-	?	+	-	?	+	-	?
	1899									
212	June 28				0	1		0	1	
583	Sept. 7				0	0	1	0	0	1
616	13				1	0		1	0	
652	20				1	0		1	0	
690	27	0	1		0	1				
1116	Dec. 20							4	0	
	1900									
1170	Jan. 4				1	1		1	0	1
1207	10							2	2	
1246	17				1	3		3	1	

TABLE X. Illinois River, Averyville.

Serial number	Date	.01 c.c.			.1 c.c.			1 c.c.		
		+	-	?	+	-	?	+	-	?
	1899									
119	June 9							0	1	
135	12							0	1	
174	21				0	1		0	1	
213	28							0	1	
387	Aug. 2							1	0	
580	Sept. 6				1	0		1	0	
617	13				0	1		0	1	
653	20				0	1		0	1	
691	27				0	1		0	1	
	1900									
1275	Jan. 24	0	4		0	2				
1312	31				0	2		2	2	
1344	Feb. 7				0	2		0	1	3
1380	14				0	0	2	3	0	1
1406	21				0	1	1	4	0	
1437	28				1	1		2	2	
1459	Mar. 7				0	2		2	2	
1498	14				1	0	1	0	2	1
1533	21				0	1	2	1	0	2
1570	28				0	2	1	3	0	
1608	April 4				0	1		1	0	1
1647	11				0	0	1	0	0	2
1687	18				0	2		0	2	
1727	25				0	1		0	1	1
1765	May 2				0	1		0	1	1
1795	9				0	1		0	1	1
1832	17				0	0	1	1	0	1
1864	23				0	1		0	0	2
1901	29				1	1		2	0	
1935	June 6				0	1		0	1	1
1969	13				0	1		0	2	
2006	20				0	1		2	0	
2041	27				0	1		0	2	

TABLE XI. Illinois River, Wesley City.

Serial number	Date	.001 c.c.			.01 c.c.			.1 c.c.			1 c.c.		
		+	-	?	+	-	?	+	-	?	+	-	?
	1899												
120	June 9							0	1		0	1	
136	14							0	1		0	1	
177	21							1	0		1	0	
214	28							1	0		1	0	
581	Sept. 6				0	0	1	1	0				
618	13				0	0	1	0	0	1			
692	27	0	1		0	1		0	0	1			
	1900												
1345	Feb. 7				0	2		1	3		0	0	2
1381	14				0	2		1	2	1	2	0	
1411	23				0	2		1	3		2	0	
1460	Mar. 7				0	2		1	1	2	1	1	
1534	22				0	2		2	0		2	0	
1581	30				0	2		0	1		1	1	
1610	April 5				0	1		0	2		1	0	
1648	11				0	0	1	0	2		0	0	1
1689	18				0	1		0	2				
1728	25				0	1		0	0	1	1	1	
1767	May 3				0	1		0	2		0	0	1
1796	9				0	1		1	0	1	1	0	
1829	16				1	0		2	0		1	0	
1866	23	0	1		0	1		1	0				
1902	31	1	0		1	0	1	1	0				
1938	June 7	0	1		0	1		0	2		1	0	
1973	14	0	1		0	1		0	2		1	0	
2008	21	0	1		1	1		1	0				
2042	28	0	1		0	2		0	1				

TABLE XII. Illinois River, Havana.

Serial number	Date	.1 c.c.			1 c.c.		
		+	-	?	+	-	?
	1899						
115	June 7				1	0	
138	14				1	0	
178	21				1	0	
221	29				1	0	
589	Sept. 7	1	0		1	0	
625	14	0	1		0	0	1
659	21	1	0		1	0	
699	29	0	1		0	1	

TABLE XIII. Sangamon River, Chandlerville.

Serial number	Date	·01 c.c.			·1 c.c.			1 c.c.		
		+	-	?	+	-	?	+	-	?
	1899									
116	June 8							0	1	
182	22							1	0	
626	Sept. 14				0	0	1	0	0	1
660	21				1	0		1	0	
715	29				1	0		1	0	
	1900									
1276	Jan. 25				1	3		4	0	
1315	Feb. 1				0	4		0	4	
1355	8				2	0		4	0	
1384	15				2	1	1	1	0	1
1438	Mar. 2				0	1	3	3	1	
1470	8				3	1		2	0	2
1508	15				1	1	1	0	3	
1544	22				3	0		3	0	
1572	29	0	2		0	2		0	1	1
1613	April 5	0	2		1	1		1	1	
1652	12	0	2		0	0	2	2	0	
1692	19	0	1		0	1		2	0	
1731	26	0	2		0	1	1	0	0	2
1770	May 3	0	1		2	0		2	0	
1805	10	1	0		2	0		1	0	
1839	17	1	0		1	0		2	0	
1875	24	1	0	1	2	0		2	0	
1911	31				1	0		1	0	
1945	June 7	0	2		0	2		1	0	
1981	14	0	1		0	2		1	0	
2011	21	2	0		0	1	1	2	0	
2051	28	0	2		0	1	1	1	0	

TABLE XIV. Illinois River, Grafton.

Serial number	Date	·01 c.c.			·1 c.c.			1 c.c.			5 c.c.		
		+	-	?	+	-	?	+	-	?	+	-	?
	1899												
117	June 8							0	1				
184	22							0	1				
222	30							0	1				
591	Sept. 7				0	1		0	1				
628	14				1	0		1	0				
662	21				1	0		1	0				
701	29				0	1		1	0				
996	Nov. 23	1	1		2	0		2	0				
1025	Dec. 1	0	1		1	0		1	0				
1057	6	0	1		0	1		1	0				

310 *Bacillus Coli Communis in River Water, etc.*

TABLE XIV. Illinois River, Grafton (*continued*).

Serial number	Date	.01 c.c.			.1 c.c.			1 c.c.			5 c.c.		
		+	-	?	+	-	?	+	-	?	+	-	?
	1899												
1098	Dec. 15				0	1		0	1	1			
1127	22				0	0	1	1	0	1			
1156	29				0	1		0	0	2			
	1900												
1187	Jan. 5				0	1		0	2				
1217	10				0	1		0	1	1			
1261	19				0	4		0	2	2			
1287	26				1	1		2	1	1			
1317	Feb. 1	0	2		0	2		2	2				
1356	8				0	1	1	2	1				
1386	15				1	0	1	2	0	2			
1408	22				0	2		3	0	1			
1484	Mar. 9				1	1		4	0				
1509	16				2	0		4	0				
1536	22				0	3		0	1	2			
1579	29				0	3		2	0	1			
1615	April 5				2	0		1	0	1			
1654	12				0	1		1	0	1			
1693	19				0	1		1	0	1			
1732	26				0	1		1	1				
1771	May 3				1	0		2	0				
1806	10				1	0		2	0				
1841	17				1	0		2	0				
1877	24				1	0		2	0				
1912	31				0	1		0	2				
1946	June 8				0	1		0	0	1	0	0	1
1983	15				0	1		1	0		1	0	
2019	22				0	1		2	0		0	0	1
2053	29				1	0		2	0		1	0	

TABLE XV. Mississippi River, Grafton.

Serial number	Date	.01 c.c.			.1 c.c.			1 c.c.			5 c.c.		
		+	-	?	+	-	?	+	-	?	+	-	?
	1899												
118	June 8							0	1				
185	22				0	1							
223	30							1	0				
592	Sept. 7				0	1		0	1				
629	14				0	1		0	0	1			
663	21				1	0		1	0				
702	29				0	1		1	0				
1026	Dec. 1	0	1		0	1		1	0				
1058	6	0	1		0	1		0	0	1			
1099	15				0	1		1	1				
1128	22				0	1		1	0	1			
1157	29				0	1		0	2				

Serial number	Date 1900	.01 c.c.			.1 c.c.			1 c.c.			5 c.c.		
		+	-	?	+	-	?	+	-	?	+	-	?
1188	Jan. 5				0	1		1	1				
1218	10				0	1		1	1				
1262	19				0	4		1	2	1			
1286	26				1	1		3	1				
1318	Feb. 1				0	2		0	2	2			
1357	8				1	1		1	1	2			
1387	15				1	1		3	1				
1409	22				0	2		2	0	2			
1485	Mar. 9				0	2		2	1				
1510	16				1	1		3	0	1			
1537	22				1	1	1	3	0				
1580	29				0	3		1	2				
1616	April 5				1	1		0	1	1			
1655	12				0	2		1	1				
1694	19				0	1		0	2				
1733	26				0	1		3	0				
1772	May 3				0	1		1	0	1			
1807	10				1	0		2	0				
1878	24				1	0		1	0	1			
1913	31				1	0		0	0	1			
1947	June 8				0	0	1	0	0	1	1	0	
1984	14				0	1		0	0	1	1	0	
2019	22				0	1		0	2		0	1	
2054	29				0	1		2	0		1	0	

TABLE XVI. Mississippi River, Cross-Section at Alton.

	Serial number	Date 1899	.1 c.c.			1 c.c.		
			+	-	?	+	-	?
East Bank	248	July 6	0	1		0	1	
	284	13	0	1		0	0	1
	319	20	0	1		0	1	
	356	27	0	1		0	1	
	736	Oct. 5	0	1		0	1	
	773	12	0	1		1	0	
	846	26	0	1		0	1	
East Centre	249	July 6	0	1		0	1	
	285	13	0	0	1	0	0	1
	320	20	0	1		0	1	
	357	27	1	0		1	0	
	737	Oct. 5	0	1		0	1	
	774	12	0	1		0	0	1
	847	26	0	1		0	1	

TABLE XVI. Mississippi River, Cross-Section at Alton (*continued*).

	Serial number	Date 1899	.1 c.c.			1 c.c.		
			+	-	?	+	-	?
Centre	250	July 6	0	1		0	1	
	286	13	0	1		1	0	
	321	20	0	1		0	1	
	358	27	0	1		0	1	
	738	Oct. 5	0	1		0	1	
	775	12	0	1		0	1	
	848	26	1	0		0	0	1
West Centre	251	July 6	0	1		1	0	
	287	13	1	0		0	1	
	322	20	0	1		0	1	
	359	27	0	1		0	1	
	739	Oct. 5	0	1		0	1	
	776	12	0	1		0	0	1
	849	26	1	0		1	0	
West Bank	252	July 6	0	1		0	1	
	288	13	0	1		0	1	
	323	20	0	1		1	0	
	360	27	0	1		1	0	
	740	Oct. 5	0	1		0	1	

TABLE XVII. Missouri River, West Alton.

Serial number	Date 1899	.01 c.c.			.1 c.c.			1 c.c.		
		+	-	?	+	-	?	+	-	?
376	July 28				0	1		0	1	
671	Sept. 23				0	1		0	0	1
714	29				0	0	1	0	0	1
749	Oct. 6				0	1		1	0	
789	13				0	1		0	1	
869	27				0	1		0	1	
	1900									
1189	Jan. 5				0	2		0	4	
1235	10				0	1		1	0	
1263	19				1	3		1	2	1
1295	26				0	2		3	1	
1320	Feb. 2				0	2		0	4	
1358	8				0	2		2	2	
1388	15				0	2		0	3	1
1410	22				0	2		0	4	
1439	Mar. 2				0	4		4	4	
1471	8				0	2		2	1	1
1499	14				2	0		0	1	2

Serial number	Date	.01 c.c.			.1 c.c.			1 c.c.		
		+	-	?	+	-	?	+	-	?
	1900									
1556	Mar. 23				0	0	1	2	0	
1594	30				2	0		2	0	
1609	April 4				2	0		2	0	
1650	11				1	0		2	0	
1690	18	0	1		0	1	1	1	1	
1721	25				0	1		2	0	
1766	May 3				0	0	1	1	0	1
1798	10				1	0		1	0	
1831	16	0	1		1	0	1			
1865	23	1	0		1	0		1	0	
1910	31	0	1		2	0		1	0	
1937	June 7				2	0		2	0	
1970	13				1	0		2	0	
2007	20	1	0		2	0		1	0	
2050	28	2	0		1	0		1	0	

TABLE XVIII. Mississippi River, Intake-Tower, St Louis
Water-works, Mitchell.

Serial number	Date	.01 c.c.			.1 c.c.			1 c.c.			5 c.c.		
		+	-	?	+	-	?	+	-	?	+	-	?
	1899												
295	July 14				1	0		1	0				
330	21				0	1		1	0				
373	28				1	0		1	0				
743	Oct. 6				0	1		0	0	1			
859	27				0	1		0	1				
	1900												
1299	Jan. 27				1	1		1	1	2			
1361	Feb. 9				1	0	1	4	0				
1426	24				1	1		1	0	3			
1474	Mar. 9				0	0	1	1	0	1			
1547	23				3	0		2	0	1			
1585	30				0	2		1	1		1	0	
1624	April 6				0	1		1	1				
1658	13				0	1		2	0				
1702	20				1	1		0	0	2			
1810	May 11				1	0		2	0		0	0	1
1845	18				0	0	1	0	0	2	0	0	1
1885	25				1	0		0	0	2			
1917	June 1				1	0		2	0				
1950	8				0	0	1	1	0	1			
1987	15				1	0		2	0				
2022	22	1	0		1	0		2	0				
2057	29	1	0		2	0		1	0				

TABLE XIX. St Louis Tap-water.

Serial number	Date	.1 c.c.			1 c.c.			5 c.c.		
		+	-	?	+	-	?	+	-	?
265	July 7	0	1		1	0				
298	15	0	1		1	0				
333	22	0	1		1	0				
365	28	0	1		0	0	1			
781	Oct. 13	0	1		0	1				
	1900									
1289	Jan. 26	1	1		1	2	0			
1347	Feb. 7	1	1		0	3	1			
1364	10	0	2		0	4				
1376	13	0	2		0	3	1			
1383	15	0	4		0	4		1		
1395	17	0	2		0	3	1			
1413	23	0	2		0	4		1	1	
1448	Mar. 5	0	2		0	3	1			
1476	9	0	1		0	1	1			
1514	17	0	1		2	0		1	0	
1549	23	0	3		2	0		1	0	
1587	30	0	2		1	1		1	0	
1626	April 6	0	1		0	2				
1660	13				0	2		1	0	
1704	21	1	1		1	1				
1743	27	0	1		0	1	1	1	0	
1778	May 4	0	1		1	1				
1812	11	0	1		2	0		1	0	
1847	18	0	1		0	0	2	1	0	
1879	25	1	0		0	2				
1914	June 1	0	1		1	1		1	0	
1952	8	0	0	1	1	1				
1989	15	0	1		1	1				
2025	23	1	0		1	0	1			
2060	29	0	1		1	0	1			

TABLE A*. Principal Stations on the Illinois River.

Collecting Stations	.00001 c.c.		.0001 c.c.		.001 c.c.		.01 c.c.		.1 c.c.	
	No. of days water exam'd	No. of days B. coli found	No. of days water exam'd	No. of days B. coli found	No. of days water exam'd	No. of days B. coli found	No. of days water exam'd	No. of days B. coli found	No. of days water exam'd	No. of days B. coli found
Ill. and Mich. Canal, Lockport	28	7	32	28	11	8	4	4	2	2
Ill. River, Morris	—	—	3	1	20	11	30	20	23	20
Ill. River, Ottawa	—	—	—	—	—	—	22	6	34	19
Ill. River, Averyville	—	—	—	—	—	—	1	0	27	4
Ill. River, Wesley City	—	—	—	—	7	1	22	3	26	13
Ill. River, Grafton	—	—	—	—	—	—	4	1	35	13

* See Note * on p. 315.

TABLE B*. Illinois River at Averyville and Grafton compared with tributaries and with the Mississippi (Grafton) and Missouri (West Alton) Rivers.

Collecting Stations	.01 c.c.		.1 c.c.		1 c.c.		5 c.c.	
	No. of days water exam'd	No. of days B. coli found	No. of days water exam'd	No. of days B. coli found	No. of days water exam'd	No. of days B. coli found	No. of days water exam'd	No. of days B. coli found
Ill. River, Averyville }	1	0	27	4	31	13	—	—
Ill. River, Grafton }	4	1	35	13	38	26	4	2
Miss. River, Grafton }	2	0	34	10	35	23	4	3
Desplaines River	—	—	8	1	5	2	—	—
Kankakee River	—	—	6	3	5	4	—	—
Fox River	—	—	22	2	23	6	13	10
Big Vermilion River	—	—	5	1	9	3	—	—
Sangamon River	13	4	25	14	27	21	—	—
Missouri River	6	3	32	13	31	21	—	—

TABLE C*.

	.1 c.c.		1 c.c.	
	No. of days water exam'd	No. of days B. coli found	No. of days water exam'd	No. of days B. coli found
Total, Illinois River (Averyville and Grafton) }	62	17	69	39
Total, tributaries of Illinois River }	66	21	69	36
Total, Mississippi and Missouri Rivers }	66	23	66	44

* It will of course be observed that this method of summarizing the results is not altogether precise. The fact that on certain days and with certain dilutions more than one determination was made obviously implies the examination of a larger quantity of water at those times and the increased possibility of a positive finding. The tabulation of the results on the basis of the total number of determinations is, however, open to objection on other grounds and the method I have employed seemed to me on the whole to present fewer disadvantages.

General Considerations.

The tables show that the number of colon bacilli found in the Illinois and Michigan Canal at Lockport is at least no greater than the number often found in fresh sewage. During the period covered by our investigation the water in the canal at Lockport, which is to be regarded as partially decomposed sewage, almost invariably revealed

the presence of colon bacilli when examined in a 1—10000 dilution and sometimes when in a 1—100000 dilution. The enormous number of colon bacilli thus indicated fairly represents the quality of the discharge into the Desplaines River at Lockport¹.

At Morris, about 27 miles below Lockport (see map) and 9 miles below the union of the Desplaines with the Kankakee, a peculiar condition exists. It has been shown elsewhere⁽⁶⁾ that the mixing of the Kankakee and Desplaines waters at Morris is very incomplete, and that the composition of the samples collected in the middle of the river at this point depends upon extremely complex and variable factors. The chlorine analyses clearly demonstrate, however, that up to the middle of January, the time when the Sanitary Canal was opened, at least one-half of the water sample from the midstream at Morris was derived from the Illinois and Michigan Canal. Since the opening of the Sanitary Canal, on the average less than one-fifth of the water sample has come from Chicago sewers. The number of colon bacilli at this point is on the whole lower than can be accounted for by simple dilution². This is particularly apparent during the later period of the investigation when the canal water constituted at least as much as one-tenth of the midstream water at Morris. During this period (Table IV., May and June) the colon bacillus was not often found in a dilution of .01 c.c., an indication that about nine-tenths of the colon bacilli originally present in the sewage had perished or in some way disappeared before reaching this point.

On the way to Ottawa, about 24 miles below Morris, the mingling of the Kankakee and Desplaines rivers becomes much more complete and some additional dilution occurs⁽⁶⁾. The weekly sample from Ottawa has contained on the average at least one-half as much water of sewage origin as the midstream sample at Morris. The number of colon bacilli found in the water at Ottawa is, again, lower than would result from simple dilution.

At Averyville, 159 miles from Chicago and just above the city of Peoria, the number of colon bacilli in the water has remained about the same throughout the whole period of the investigation, and has

¹ The volume of water in the Desplaines River is insignificant at most seasons, and but for the discharge from the canal the river bed would be nearly dry for three-quarters of the year.

² It must be noted also that the sewage of Joliet (population, 29,353, according to the U. S. Census for 1900) contributes its quota of fresh colon bacilli to the river between Lockport and Morris.

averaged quite as low as the number found in the various tributaries of the Illinois and in river waters in general (cf. Table B).

Immediately below Peoria (Wesley City, Table XI.) the number of colon bacteria is increased as the natural consequence of the influx of the sewage of Peoria. At the mouth of the Illinois River (Grafton, Table XIV.) the number of colon bacteria ranges slightly higher if anything than at Averyville, a condition that may perhaps be accounted for in part by the influence of the Sangamon River, which has had a persistently high colon content during the period covered by our analyses. The actual number of determinations is not large enough, however, to admit of much stress being laid upon the apparent divergence between Averyville and Grafton.

It is not intended in this paper to assume that no change occurs in the gas-producing power of the colon bacillus during its sojourn in the river water. All that the results prove is that those varieties of colon bacilli which happen to be revealed by the methods used and are characterized by the gas-producing qualities elsewhere described, disappear from the flowing stream. Whether this is due wholly to the death and sedimentation of this class of bacteria or in part also to a modification of individual cells with reference to their aerogenic power is a question too recondite to be discussed in the light of our present imperfect knowledge of the causes modifying gas production. It is quite possible that some colon bacilli may become so disguised by prolonged aquatic life as to be no longer recognizable by the methods used. It must be recalled, however, that it is always possible to recover colon bacilli possessed of typical gas-producing qualities from sewage and from polluted river waters that have been stored for some weeks in glass bottles in the laboratory. In one instance we have found colon bacilli yielding typical gas production in a 1—1000 dilution of sewage that had been standing in a bottle for forty-two days, and in another case we have found them six months after the sewage had been collected.

No indications have been noted that the *season of year* bears any very marked relation to the number of colon bacteria in the river waters examined. There appears, however, to be a slight increase in some rivers during the months of February and March when the streams are swollen by heavy rains and melting snow. On the supposition that the exhaustion of food-supply or the accumulation of harmful metabolic products is the primary cause of the disappearance of the colon bacilli and other sewage bacteria from flowing streams, the effect of flood-time

is easy to understand. It is not simply that the bacteria are prevented by a more rapid current from settling out, but that they, *together with their food-supply*, are swept along over a greater distance in the same period of time. The actual length of time that the food-supply holds out may or may not be the same at high water as at low water, but the distance traversed is certainly greater in the former case. The same reasoning is applicable in case the heaping up of injurious substances as the result of bacterial metabolism is the lethal cause.

The possible influence of the opening of the Sanitary Canal upon the colon content of the Illinois River is a matter of considerable interest. The connection between the river and the canal was first broken through on Jan. 1, 1900, and although the controlling gates at Lockport were not opened until Jan. 17, the composition of the water pumped from the Chicago River into the Illinois and Michigan Canal at Bridgeport was changed immediately by the inrush of purer water from Lake Michigan. At Morris and Ottawa such slight alteration in the colon content as can be observed is in the direction of smaller numbers, as should indeed be anticipated on the ground of greater dilution. At Averyville no significant difference between the two periods can be noted. There is no evidence that the number of colon bacilli in the water at the mouth of the Illinois River, 160 miles below Averyville, was affected by the opening of the Sanitary Canal. During the period under consideration, in fact, the streams tributary to the Illinois contained about the same number of colon germs as did the Illinois River at Averyville.

For the sake of comparison it will be useful to cite here the more important observations that have been made regarding the presence of *B. coli communis* in the river waters of the United States. Smith and Brown⁽⁸⁾ state that "The numbers of *Bacilli coli communis* found in water supplies from sources considered on inspection to be unpolluted, vary from nine to five [per cubic centimetre]. The numbers in Mississippi River water as supplied to St Louis vary from three to seven, and the numbers in Hudson River water at the intake of the Albany Water-works, vary from twenty to over ninety on different stages of the tide." It is interesting to note that if we employ the method of enumeration used by these authors, the number of colon bacilli which we have found in the Mississippi River at the intake tower of the St Louis Water-works would be stated as being on the average five per cubic centimetre (Table XVIII. column of '1 c.c.), and the number in the water-supply as delivered to the consumer as one per cubic

centimetre (Table XIX.), the latter figure being exactly the same as the number in the Illinois River at Averyville (Table X.).

Fuller⁽³⁾ has recorded the finding of *B. coli communis* in the Ohio River water at Cincinnati between March 24 and December 29, 1898. Out of 197 determinations on different days a positive result was reached in 114 or about 58%. This is very close to the result reached for the Illinois River (Table C.), which, put into percentage form, gives 56% of positive results for the one cubic centimetre determinations. The number of colon bacilli in the Ohio River appears to have been somewhat greater during the summer than during the winter months.

Clark⁽⁴⁾, who used, however, the less delicate plate method of enumeration, records an average of 47 colon bacilli per cubic centimetre in the Merrimack River water at the filter intake of the Lawrence City Water-works. In 1898 the range was from 20 per cubic centimetre in May and June to 92 in August and September; in 1899, from 8 in August to 140 in December.

It is perhaps needless to dwell upon the peculiar value of systematic observations regarding the presence and abundance of the colon bacillus in all studies of the pollution and self-purification of streams. The life-history of the colon bacillus in flowing water brings us closer to the real problems at issue than observations of any other nature. Under ordinary conditions the chief danger from sewage-polluted surface water in this country lies in the possible presence of the typhoid bacillus and perhaps of one or two other closely related organisms. Since there are no methods in common use that are universally applicable to the direct detection of these microbes, the fate of typhoid germs introduced into a stream or lake must be determined by inference. Now it is a far cry from the "chlorine," "free ammonia," and "nitrates" in a water to its content of typhoid germs, and the actual number of bacteria of all kinds in a water is a criterion of but slightly greater value. The colon bacillus, however, is biologically similar to the typhoid germ, and like the latter its presence in sewage is due almost wholly to human life and habitation. There is specific reason to believe that the duration of life of the colon bacillus in water is considerably greater than that of the typhoid bacillus, a belief that is in accord with what is known regarding the more robust character of the former organism. The best index we can secure at present, therefore, for gauging the probable continuance of vitality of the typhoid bacillus in running water is the information derived from the fate of the colon bacillus. If this nearly of the typhoid bacillus perishes speedily and in large numbers in a

given stretch of river there is good reason to suppose that the typhoid bacillus itself will not survive exposure to the same conditions. The rate of mortality among colon bacteria probably indicates more surely than any other factor the death-rate among typhoid bacilli under a similar stress. Wherever we find that an extensive mortality occurs among colon bacteria, in the present state of our knowledge we are justified in assuming that the fatality among typhoid bacteria has been at least equally great.

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ON THE INFLUENCE OF FORMIC ALDEHYDE UPON THE METABOLISM OF CHILDREN.

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FORMIC aldehyde, the simplest possible aldehyde, is a substance possessed of considerable biological and chemical interest. Its biological interest consists in the fact that it has long been regarded as forming the first product of plant assimilation, and has recently been actually demonstrated as such in plants¹. The ease with which this substance undergoes polymerisation resulting in the formation of sugars, under the influence of simple reagents, justifies the assumption that formic aldehyde is an essential link in the building-up of complex carbohydrates by plants. In addition to this it has recently been requisitioned as the only possible source of proteid anabolism². Its interest to the physiological chemist is that it combines energetically with proteids, and to a less extent with carbohydrates, effecting considerable alteration of their physical and physiological properties. This behaviour seems to be directly antagonistic to the biological rôle ascribed to the substance above. This apparent anomaly of the same substance being both a poison and an essential physiological constituent of living cells is discussed by Loew³. It seems to depend upon the fact

¹ Pollaci, *Boll. chim. farmac.*, 1899, xxxviii. p. 1601.

² Loew, *Die chemische Energie d. lebenden Zellen*, pp. 67, 86, etc. and R. Hebert, *Ann. Argonom.* xcvi. p. 416.

³ *loc. cit.* p. 66.

that what may be termed the biological formic aldehyde is present in small quantities (dilute solutions) and is quickly used up, owing to the ease with which it polymerises or forms innocuous compounds. This last property has an immediate bearing upon the subject of this inquiry in so far as it may serve to explain the results obtained by the administration of small quantities of formic aldehyde to man in his food. It is in this connection that formic aldehyde is of interest to us, and the importance of this subject is in our opinion sufficient to justify us in reviewing somewhat exhaustively the results of other observers with regard to the chemical and physiological properties of this substance.

Hofmann¹ discovered formic aldehyde (CH_2O) in 1869. It is a gas, soluble in water, and obtainable commercially as a 40 % solution which is known as formaline or formol. It has an irritating and pungent odour, and is easily converted into a polymeric solid modification. Its disinfectant action was first pointed out by Loew² and has subsequently engaged the attention of numerous workers, amongst whom may be mentioned Trillat, Aronson, Blum, etc. Their results are mostly of interest to bacteriologists, and are outside the subject of our inquiry. The literature so far as it concerns us may be classified as follows: (I) chemical action of formic aldehyde upon proteids, (II) influence of formic aldehyde upon digestions *in vitro*, (III) experiments upon animals.

Chemical Action of Formic Aldehyde upon Proteids.

The action of formic aldehyde upon native proteids is, in general, to render them insoluble. Egg and serum albumin are however exceptions. The compounds which formic aldehyde forms with them are soluble and non-coagulable by heat³. The reaction between formic aldehyde and the proteids is explained by assuming an interaction between the aldehyde and the amido groups of the proteid molecule: there is however no proof of this. The insoluble compounds are resistant to

¹ *Berichte d. deutsch. chem. Ges.* 1869, p. 152.

² Loew, *München. med. Wochenschr.*, 1888, p. 412.

³ In this regard it may be mentioned that Trillat (*Compt. rend. Acad. d. Sc. Paris*, 1892, T. cxiv. p. 1279) states that formic aldehyde coagulates albumin as well as blood. Subsequently Blum (*Zeitschr. für physiol. Chemie*, 1896, p. 137) found that formic aldehyde prevents the coagulation by heat, of serum and egg albumin in aqueous solution, a fact which has been confirmed by Benedicenti and all later observers. See also L. Schwarz, *Zeit. f. phys. Chemie*, 1901, xxxi. p. 460.

the action of the digestive enzymes¹. By the action of steam the formic aldehyde is easily split off, the proteids reformed possessing their original digestibility. (Blum², Bach³, Benedicenti⁴, Beckmann⁵, Mosso and Paoletti⁶, Foulerton⁷, Riche⁸.)

According to Lepinois⁹ a 1% solution of formic aldehyde preserves thyroid glands without affecting either their digestibility or the solubility of the active principle—(iodo-thyrin).

The evidence concerning the action of formic aldehyde upon albumoses and peptones is somewhat conflicting¹⁰.

According to Beckmann¹¹, gelatine peptone and egg albumin peptone are unaffected by formic aldehyde, whereas hemialbumose is rendered insoluble. Trillat without giving any experimental data makes the statement that formic aldehyde renders insoluble all proteid substances not coagulable by heat. This generalisation would of course include albumoses and peptones¹². Lepierre¹³ worked on the action of formic aldehyde upon the products of partial peptic digestion isolated by himself, viz. hetero-, proto-, and deutero-albumoses, and peptones, also upon commercial peptones the composition of which he previously examined in the usual manner. According to him there was no action in the cold, and even at 100° C., working with a 14% formic aldehyde solution, only in some cases did precipitation take place, usually (commercial peptones) no change occurred. From subsequent examination of the resulting solutions, concerning which no details are given, he enunciates a theory according to which formic aldehyde causes a "progressive regression" of the simpler products of proteid digestion back again to the more complicated ones, viz. of peptones to deuterio-albumoses, of deuterio-albumoses back to proto-albumoses, which finally

¹ Hehner (*Analyst*, xxii. 1897) said in a discussion on formic aldehyde that he had seen the results of certain experiments which showed that the soluble compound formed by formic aldehyde and egg albumin was digested more easily than egg albumin itself.

² *Zeitschr. f. physiol. Chemie*, 1896, xxii. p. 137.

³ *Moniteur Scientifique*, xi. p. 157.

⁴ *Du Bois-Reymond's Archiv*, 1897, p. 210.

⁵ *Forschungs-Berichte über Lebensmittel-Unters.*, etc., iii. p. 324.

⁶ *Giorn. d. r. Accad. di med. di Torino*, 1895.

⁷ *Lancet*, ii. 1899, p. 1579.

⁸ *Journ. Pharm. Chim.* vi. p. 197.

⁹ *Bull. Soc. Chim.*, 1899, T. ix. p. 76.

¹⁰ Loew (*loc. cit.*) observed that pure peptone gave no precipitate with formic aldehyde, whilst commercial peptones (Witte) yielded immediately a precipitate in the cold; this he regards as being due to the presence of albumoses (propeptone).

¹¹ *loc. cit.*

¹² *Bull. Soc. Chim.* 1898, p. 1017.

¹³ *Compt. rend. Acad. d. Sc. Paris*, T. cxxviii., p. 789.

are precipitated. This observer states further that the compounds formed by formic aldehyde and the proteids are digestible, but less rapidly than the corresponding original proteid, and that these compounds could be reconverted into the original easily digestible proteids by the action of superheated steam.

Influence of Formic Aldehyde upon Digestions in Vitro.

The work done in this connection may be divided into that which concerns itself with the action of the digestive enzymes in the presence of formaldehyde, with their action upon formalised food, and with the effect of formic aldehyde upon the enzymes themselves. The digestions can be considered in this regard *seriatim*.

Salivary Digestion. Rideal and Foulerton¹ estimated the amount of maltose formed from arrow-root starch by saliva alone and in the presence of formic aldehyde in different proportions. Taking the control as 100, they found that ptyaline in the presence of 1 in 100,000 formic aldehyde converted 99·8 %, in the presence of 1 in 10,000, 89·0 %. Foulerton² tested the progress of starch conversion in the presence of formic aldehyde and found that dilute solutions, 1 in 40,000 to 1 in 1,000, had a retarding but not an inhibitory action.

The relative convertibility of previously formalised starch into sugar by means of ptyaline has so far as we are aware only been investigated by Bliss and Novy³. These observers found that a 1 % starch paste previously kept for 5 days at 35° C. with formic aldehyde (1 in 100) behaved with ptyaline exactly as fresh starch paste.

The same investigators showed that formic aldehyde in the strength of 1 in 1,000 exerts very little action upon ptyaline itself, unless the mixture is allowed to stand for several days or is kept at 35°—40° C. With stronger solutions of formic aldehyde the effect is more marked, the ferment being destroyed by the action of 1 in 1,000 for 9 days at 40° C.

Rennet. Pottevin⁴ observed that formic aldehyde added to milk retarded its coagulation by rennet, and that rennet itself (*loc. cit.*) was rendered inactive by strong solutions of formic aldehyde.

¹ *Public Health*, 1899, p. 554.

² *Lancet*, 1899, II. p. 1432.

³ *Journ. of Experimental Med.*, 1899, IV. p. 74.

⁴ *Ann. de l'Inst. Pasteur*, 1897, p. 807.

Bliss and Novy also examined the action of rennet upon formalised milk and found that milk which had been subjected to the action of formic aldehyde, 1 in 1,500, behaved in this respect very similarly to normal milk. (Normal milk coagulated in 15 minutes, this formalised milk in 20 minutes.) Milk which had been acted upon by formic aldehyde, 1 in 1,500 for 1 hour, behaved identically with normal milk.

Foulerton's¹ experiments with rennet show that formic aldehyde added to milk in the proportion of 1 in 40,000, whether at the same time as the enzyme or 48 hours before, has practically no influence upon the process of coagulation. With higher proportions up to 1 in 5,000 coagulation was retarded, but not prevented. This was also the case to a more marked degree with solutions of 1 in 1,000. These results with higher proportions do not correspond with those of Bliss and Novy detailed above. The discrepancy in the results of these two observers is probably to be explained by the variations in milks and rennets used. It will be noted that the behaviour of the control milks was not identical (coagulation in 15 minutes Bliss and Novy, and 30 minutes Foulerton).

Halliburton² observed that strong solutions of formic aldehyde delayed rennet action, more dilute solutions acted similarly but to a less marked degree. Freudenreich³ observed that formic aldehyde in the form of vapour had a destructive influence upon rennet, but that in solution in the strength of 1 in 500 it had no appreciable action upon its milk-coagulating power.

With regard to the action of formic aldehyde upon the rennet ferment itself Bliss and Novy⁴ found that it had no apparent effect upon this ferment, even when present in the proportion of 1 in 50.

Peptic Digestion. Symons⁵ found that formic aldehyde did not influence peptic digestion.

According to Mayberry and Goldsmith⁶, when pepsin was allowed to act upon fibrin in the presence of formic aldehyde the amount of fibrin digested diminished with the increasing percentage of

¹ *Lancet*, 1899, II. p. 1578.

² Halliburton, *Brit. Med. Journ.*, 1900, II. p. 2.

³ Freudenreich, *Centralbl. f. Bakteriologie*, Abth. II. 1898, p. 309.

⁴ *loc. cit.*

⁵ *Journ. of American Chem. Soc.*, 1897, XIX. p. 744.

⁶ *Ibid.* p. 889.

formic aldehyde. Taking the control as 100 they found that, when formic aldehyde was present in the proportion of 1:2,000, 97·74% of the fibrin was digested, when present in the proportion 1:1,000, 94·34%¹.

Rideal and Foulerton² concluded from carefully executed quantitative experiments that the addition of formic aldehyde immediately before digestion in the proportion of 1:50,000 had no appreciable effect. Their quantitative results were as follows. When formic aldehyde was added in the proportion of 1:50,000 immediately before digestion, the amount digested in unit time was 97·63%, taking the control as 100.

With regard to *previously formalised* proteid they found that (taking again the control as 100) 91·45% was digested in the case of fresh beefsteak previously formalised for 24 hours with 1:100,000, 90·38% when the strength used was 1:50,000, 85·25% when the formic aldehyde was increased to 1:10,000. From these results they conclude that formic aldehyde has no influence on the digestibility of the food after contact with it for 24 hours prior to the action of the enzyme.

These conclusions have been criticised by Hehner³ and in a subsequent paper Foulerton⁴ modifies them considerably, and infers that in addition to any possible effect which formic aldehyde may have on the action of the enzyme, it also renders food itself less digestible.

Bliss and Novy⁵ found that the digestion of fibrin by pepsin, both previously formalised for 24 hours, proceeded normally when the strength of formic aldehyde was 1:2,500, and even when it was increased to 1:100 the fibrin was eventually digested.

Halliburton⁶ made a series of experiments on the relative digestibility of fibrin previously treated with formic aldehyde (for from two to three days with 1:100 to 1:2000). He found that previous formalisation with 1:2,000 for two days delayed gastric digestion 20 minutes, for three days for 30 minutes. Formalisation with a 1% solution did not prevent digestion, the latter becoming complete in 24 hours.

¹ The results of Mayberry and Goldsmith were re-calculated by us, from the average of their experiments (taking the average of the three control experiments as 100).

² *Public Health*, 1899, p. 554.

³ *Brit. Food Journ.*, 1899, p. 132.

⁴ Foulerton, *Lancet*, 1899, pp. 1432 and 1577.

⁵ *loc. cit.*

⁶ *Brit. Med. Journ.*, 1900, II. p. 2.

Loew¹ was the first to study the action of *formic aldehyde on pepsin* and found that when this enzyme was exposed to strong solutions for one day it lost its activity.

Bliss and Novy² subsequently found that pepsin is not affected by a 1% solution of formic aldehyde, even when the mixture has stood for four weeks. Even a 5% solution acting for three weeks has no effect on pepsin. Contrary results by others are explained as being due to an alteration of the fibrin by the aldehyde, before the pepsin could act.

Pancreatic Digestion. Symons³ found that when formic aldehyde was added to the digestive mixture in the proportion of 1:2,000, it had a distinctly retarding influence on pancreatic proteolysis (fibrin), whilst 1:300 completely inhibited digestion.

Rideal and Foulerton's⁴ results showed that when formic aldehyde was added in the proportion of 1:50,000 to milk immediately before its artificial digestion with commercial pancreatin 97.0% of casein was digested, taking the amount digested in the control experiment as 100.

With regard to *previously formalised* milk the same authors found that 94.1% casein was digested when milk formalised to the extent of 1:50,000 was acted upon by commercial pancreatin (taking as before the amount digested in the control as 100).

Bliss and Novy working with commercial pancreatin and fibrin found that fibrin previously formalised for 24 hours with 1 in 1,000 formic aldehyde was digested much more slowly than fresh fibrin, but that its total digestion did eventually take place. The same observers working with *fresh pancreatic extract* found that when fibrin was formalised for 24 hours with 1 in 1,000 there was no influence upon its digestibility, whilst when 1 in 500 was used there was a slight retarding influence.

Halliburton⁵ studied the effect of previous formalisation upon the digestibility of fibrin by means of commercial pancreatin and found that whereas the digestion of fresh fibrin was completed in 30 minutes that of fibrin previously formalised by an exposure to formic aldehyde solution 1 in 2,000 for two days required 95 minutes for its completion. In the case of formalisation with stronger solutions no signs of digestion occurred within 24 hours.

Loew⁶ observed that when a *solution of trypsin* was treated with

¹ *loc. cit.*

² *loc. cit.*

³ *loc. cit.*

⁴ *loc. cit.*

⁵ *loc. cit.*

⁶ *loc. cit.*

formic aldehyde a precipitate occurred. The action of formic aldehyde upon the pancreatic enzyme has been carefully studied by Bliss and Novy¹. They found in the case of commercial pancreatin (Parke Davis) that solutions of formic aldehyde in the strength of from 1 in 1,000 to 1 in 100 acting for 24 hours completely destroyed its proteolytic activity. In the case of freshly prepared pancreatic extract the influence was not so marked, a strong extract, previously formalised for 24 hours, with 1 in 1,000, digested formalised fibrin (1 in 1,000) normally; when the ferment solution was previously formalised with 1 in 500 there was a distinct retardation in the case of the *strong extract*, and *absolute destruction of proteolytic power* in the case of the weak extract.

Amylolytic Ferment of Pancreas. Rideal and Foulerton² tested the activity of the amylolytic ferment of two samples of commercial pancreatin in the presence of formic aldehyde. They found that when formic aldehyde was added immediately before digestion, so that it was present in the digestive mixture in the proportion of 1 in 50,000, 91.8% maltose was produced in the case of Extract No. I and 84.00% in the case of Extract No. II. When the formic aldehyde was present in the proportion of 1 in 10,000, 91.5% and 83.0% maltose were produced respectively. The amount produced under normal conditions was taken as 100.

Halliburton³ made a few experiments with regard to starch conversion by means of commercial Liq. Pancreaticus (Benger) in the presence of formic aldehyde and found that the strongest solution of formic aldehyde caused a retardation of total conversion amounting to five minutes, the weakest a retardation amounting to two minutes. Bliss and Novy⁴ made some observations upon the amylolytic power of previously formalised freshly prepared pancreatic extracts. From their results it follows that formic aldehyde has very little influence upon them. Amylopsin previously formalised for 24 hours with 1 in 1,000 was not affected, even 1 in 500 exerted but little action⁵.

Experiments upon Animals.

The symptoms produced by formic aldehyde consist in strong local irritation. The animals become restless and exhibit clonic convulsions and opisthotonus. They finally pass into a condition of coma, during which the respiration is quick and irregular in rhythm, the cause of

¹ *loc. cit.*

² *loc. cit.*

³ *loc. cit.*

⁴ *loc. cit.*

⁵ "Lipase" seems unaffected by formic aldehyde. Kastle and Loewenhardt, *Am. Chem. Journ.* xxiv. No. 6.

death being asphyxia. According to Benedicenti¹ formic aldehyde is a blood poison, converting haemoglobin into haematin.

The toxicity of formic aldehyde upon higher animals seems to be relatively low and differs considerably according to the animal.

Trillat² gives the lethal dose per kg. body weight for guinea-pigs as 0.8 g. when injected *sub cutem*, whilst 0.53 and 0.66 produced no effect; 0.038 g. per kg. guinea-pig, injected into a vein was also without result.

Berlioz and Trillat³ found the toxic dose for dogs (intravenous) to be 0.07 g. pro kg. and for rabbits (intravenous) 0.09 g. pro kg.

Aronson⁴ found 0.24 g. pro kg. body weight to be lethal for rabbits, and according to Pottevin⁵ larger doses than 0.25 g. pro kg. *sub cutem* and 0.03 g. pro kg. intravenous are lethal for the same animal. 0.016 g. injected into the veins of rabbits for four days in succession produced no effect.

According to Mosso and Paoletti⁶ 0.32 g. pro kg. proved lethal for dogs, whilst 0.22 g. produced serious symptoms.

Bruni⁷ found that 0.28 g. pro kg. killed a dog to which the same dose had been administered *per os* the day before.

When administered by the mouth the results obtained are somewhat conflicting.

Blum⁸ gave to a rabbit weighing 1,500 g. 0.72 g. formic aldehyde in two doses in the form of a 1.2 % and of a 2.4 % solution. The animal refused food for a day. He also administered 1.5 c.c. of a 40 % formalin solution, that is 0.6 g. formic aldehyde, to a hare. The hare took its food normally after two days.

U. Mosso and Paoletti⁹ gave to a dog 0.04 g. formic aldehyde in the form of a 1 in 250 solution. Vomiting ensued. Subsequently they gave 0.022 g. formic aldehyde in the form of a 1:500 solution. No vomiting occurred, but symptoms referable to an action of the drug upon the central nervous system. A dose of 0.011 g. in the same dilution produced no effect.

Bruni¹⁰ found that 0.032 g. of a 1:1,000 solution had no effect upon a dog weighing 7.2 kg., and that 0.28 g. formic aldehyde in the form of

¹ *Arch. f. (Anat.) Phys.*, 1897, p. 210.

² *loc. cit.*

³ *Compt. rend.* 1892, T. cxv. p. 291.

⁴ *Berliner klin. Wochensch.* 1892, p. 751.

⁵ *Ann. de l'Inst. Pasteur*, 1894, p. 807.

⁶ *Giorn. d. r. Accad. di med. di Torino*, 1895.

⁷ *Ann. di Farmacoterapia e Chimica*, 1899, p. 339.

⁸ *Münchener med. Wochensch.* 1893, p. 602.

⁹ *loc. cit.*

¹⁰ *loc. cit.* p. 338, et seq.

1:500 solution caused vomiting in a dog weighing 14 kgs. After 50 minutes no further disturbances occurred.

Some experiments have been made with regard to the general effect of doses of formic aldehyde continued for some time upon animals.

Rideal and Foulerton¹ fed cats three months old for a period of several weeks on milk containing formic aldehyde to the extent of 1 in 50,000, 1 in 25,000, and 1 in 20,000, and in two cases noted an increase in weight, while in one case the weight remained constant. They further fed one rabbit in the same way, estimating the amount of nitrogen in the food and the excretions, and found that the animal gained in weight and that although there was some increase in the excretion of nitrogen, the nitrogen balance remained positive. These observers however give no control experiments. Reviewing these experiments in a later article² Foulerton concludes that these observations were not sufficiently numerous to allow of any stress being laid upon them either one way or the other.

Annett published some experiments³ on young kittens three or four weeks old extending over several weeks. These he fed on milk containing formic aldehyde 1 in 50,000, 1 in 25,000, and 1 in 12,500. Control experiments were made on a smaller number of kittens, fed on normal milk. The quantity of milk taken by each kitten is not given, therefore no data are available with regard to the quantity of formic aldehyde consumed by each kitten. These experiments and the conclusions drawn from them have been criticised at length by Liebreich⁴ and Rideal⁵, who have pointed out that the results were so irregular⁶ in comparison to the number of animals experimented upon, that no important conclusions could be drawn from them.

Rideal further pointed out that cows' milk as such is an unsuitable food for kittens of that age and that six kittens fed on undiluted fresh cows' milk all died. Rideal in the same paper published a series of observations upon kittens five weeks old fed with 70 c.c. of cows' milk per diem formalised to the extent of 1 in 5,000; he noticed under these conditions no injurious action but an increase in body weight.

In this connection some experiments have been made by A. D. Hall and H. S. Hammond in collaboration with ourselves at the South Eastern

¹ *loc. cit.*

² *Lancet*, 1899, II. p. 1582.

³ *Lancet*, 1899, II. p. 1284.

⁴ Liebreich, *Lancet*, 1900, I. p. 13.

⁵ Rideal, *Lancet*, 1900, I. p. 228.

⁶ *E.g.* he obtained three deaths in the case of the 1 in 50,000 milk, none with the 1 in 25,000, and two with the 1 in 12,500.

Agricultural College, Wye. These observations will be published fully elsewhere; they showed that the continuous administration for six weeks of from .8 to 1.6 grammes daily of formic aldehyde with a mixed diet had no effect upon the live weight of young sucking-pigs. The initial weight of the pigs varied from 25 to 58 lbs., and the quantity of formic aldehyde given amounted to a concentration of from 1 in 185 to 1 in 730 of the total food.

Concerning *the fate of formic aldehyde* in the animal body the results of various observers are not in accord.

Trillat (*loc. cit.*) found that guinea-pigs' urine underwent no fermentative change after they had been injected with formic aldehyde. According to Aronson (*loc. cit.*) the urine of rabbits after the administration of large doses of formic aldehyde has a reducing action upon ammoniacal silver nitrate and gives Schiff's reaction for aldehydes. Blum (*loc. cit.*) could not find formic aldehyde in the urine of rabbits after doses of 0.6 g., but obtained traces of formic acid.

Quite recently Filippi and Martoleni¹ have studied the fate of formic aldehyde in the body after injection. The object of the research seemed to be to determine whether the results of Albertoni² concerning acetic aldehyde, namely, its complete excretion as such without oxidation, were true for formic aldehyde. They seem unaware of the fact that these results have not been confirmed by Reizenstein³. They quote Perando⁴ as having found that formic aldehyde is completely oxidised in the body, to formic acid, and call into question his methods. From their own experiments they conclude that formic aldehyde after its administration in apparently very large doses could be found in all the organs of the body; the colour reaction upon which they relied being most marked in the intestines, lungs and kidneys. No mention is made as to its presence in the urine.

Among the compounds of formic aldehyde easily decomposable into the original substance, Pohl⁵ has examined the fate in the body of the sodium sulphite compound $\left(\text{HCH} \begin{array}{l} \text{OH} \\ \text{SO}_3\text{Na} \end{array} \right)$. After the administration of 5 g. of this substance to a dog, no aldehyde reaction could be obtained in the urine, but there was a very slight increase in the amount

¹ *Ann. Farmacoterapia e Chimica*, 1900, p. 195.

² *Albertoni e Lussana*, Padua, 1875. Albertoni, *Arch. Italiane de Biologie*, 1888.

³ *Dissertation*, Würzburg, 1894.

⁴ Perando, *Boll. di R. Acc. med. di Genova*, Vol. xi. No. 9.

⁵ *Arch. f. exper. Path. u. Pharm.*, 1893, p. 281.

of formic acid normally present. The fate of the ammonium compound of formic aldehyde (hexamethylentetramine or urotropin) is discussed by Nicolaier¹ in his original communication. His results have subsequently been confirmed by other observers. Urotropin appears rapidly in neutral or alkaline urines as such, and in acid urines as formic aldehyde.

With regard to *the fate of formic aldehyde* in the body the observations of Pohl (*loc. cit.*) are of interest. He found that the fresh organs of warm-blooded animals, especially the liver, can oxidize formic aldehyde to formic acid. Jacobi² isolated a pure ferment from the liver of oxen, possessing very strongly the power of oxidizing aldehydes, especially salicylic aldehyde.

Action on Man. Very little is known concerning the action of formic aldehyde on man, either in therapeutic or poisonous doses. A case of poisoning is recorded by Andés³ in which a "spoonful" of a 40% formic aldehyde solution was taken by mistake. Ammonium acetate and an emetic were administered immediately and complete recovery took place in two days. No general symptoms other than those referable to shock were recorded.

Another case is mentioned in the *Medical Press*⁴, where a youth took about 2.5 g. of formic aldehyde in the form of a 4% solution. Vomiting occurred and death 29 hours afterwards from heart-failure. A post-mortem examination showed "that the oesophagus was slightly inflamed and escharotic changes were visible in the stomach."

Trillat⁵ gave 5 g. of the polymerised solid modification of formic aldehyde to patients without any poisonous symptoms. Aronson records similar experiences with paraformaldehyde.

The ammonium compound (urotropin) mentioned above is used extensively in medicine.

Quite recently⁶ 50 c.c. of a 1 in 2,000 solution of formic aldehyde have been injected intravenously as a means of treatment in pulmonary tuberculosis, without any symptoms of poisoning occurring. The injections were continued over a considerable time.

¹ *Centralbl. f. med. Wiss.*, 1894, p. 897, and *Deutsche Med. Wochenschr.*, 1895, p. 541.

² *Zeitschr. f. physiol. Chemie*, 1900, p. 133.

³ *Journ. de Pharm. et Chim.*, 1899, T. x. p. 10.

⁴ *Medical Press*, 1899, p. 309.

⁵ *Journ. de Pharm. et Chim.* 1894, p. 540.

⁶ Reported in *Brit. Med. Journ.* from Dr Maguire's *Harveian Lectures*, Nov. 24th, 1900.

Critical Review of Literature.

In this connection we do not purpose considering formic aldehyde from either a therapeutical, bacteriological or toxicological standpoint, but from the standpoint of its possible use as a food-preservative. This limitation of our point of view brings us at once to a limitation of material and quantity. Material in so far as we have only to consider milk, and quantity in so far as only those experiments are relevant in which formic aldehyde is used in quantities not in excess of those necessary for its action as a preservative. In this regard the strong solutions are at once excluded owing to their taste, and because they are unnecessary¹.

From a careful consideration of the results of various workers we are inclined to accept 1 in 25,000² as the maximum quantity which can possibly come in regard in this connection, but as it will be seen from the context we took for the purpose of our own experiments a higher proportion (1 in 10,000 and 1 in 5,000).

If we consider the literature, keeping these limitations in mind, the following points are to be emphasised:—

From the experiments made to ascertain the chemical action of formic aldehyde upon the proteids generally, the conclusion may be drawn that compounds of a more or less definite composition are formed, and that the digestibility of these compounds is less than that of the original proteids. All these experiments however relate to compounds produced by the action of an excess of formic aldehyde, none being recorded with dilution approximating to that of the above adopted standard. Taking in regard the complex nature of milk and the excess of chemical compounds capable of combining with formic aldehyde in it, in proportion to the very small amount of formic aldehyde added, it does not seem to be justifiable to draw *a priori*

¹ By the assistance of a number of observers we were able to convince ourselves that formic aldehyde in the strength of 1 in 2,000 imparted to milk in the cold a characteristic taste. If the milk were warmed to drinking temperature 1 in 5,000 could be easily detected.

² Vide Droop Richmond and Harrison, *Analyst*, 1900, p. 116.

It is further interesting in this connection to observe, assuming Benedicenti's figures to be correct (*loc. cit.* p. 243), that milk could fix owing to its 3% of casein 0.0036% formic aldehyde, whilst 1 in 25,000 formic aldehyde corresponds to 0.004% . There seems to be a relation between the fixing of the formic aldehyde by the casein and its power as a preservative as the time during which this amount will keep milk sweet corresponds approximately to the time required for the proteid to fix all the formic aldehyde.

conclusions as to the effect of formic aldehyde upon the digestibility of the proteid constituents of milk, from the mere chemical data above.

With regard to the chemical reactions between formic aldehyde and the products of partial or complete proteid digestion, we must bear in mind again that so far as we know these reactions only take place at unphysiological temperatures¹, and with relatively concentrated solutions of formic aldehyde.

If we direct our attention to the digestion experiments *in vitro* with formic aldehyde, we come to the conclusion that speaking generally under the conditions of these experiments, formic aldehyde has a retarding effect upon the digestion of food by the various enzymes concerned. In the case of peptic digestion this effect is less marked (according to some observers there is no effect) than in the case of pancreatic. The general value of these conclusions is however in our opinion somewhat lessened by the following considerations. All experiments have been made with commercial pepsin or trypsin, except the later pancreatic digestions of Bliss and Novy, from which it is manifest that the difference between the digestibility of formalised and fresh proteid is far less marked in the case of fresh trypsin than in that of commercial. These observers found that, in the case of freshly prepared pancreatic juice, fibrin formalised with 1 in 1,000 formic aldehyde was as digestible as fresh fibrin. Since we have no experimental data with regard to fresh pepsin we cannot tell how this latter would have behaved in the presence of formic aldehyde.

No digestion *in vitro* following physiological sequence has been carried out, in no case has the residue of the gastric digestion of a formalised food been subjected to pancreatic digestion. No quantitative experiments as far as we are aware have been made concerning the most important point under consideration, viz. the gastric digestion of formalised milk.

It has been pointed out by many critics² that conclusions from experiments *in vitro* can only be applied to living animals if at all with great caution, since the conditions which obtain in the living stomach are much more complicated, and impossible to imitate *in vitro*. To mention one of the more gross objections we may point out that no attempt is made in experiments *in vitro* to imitate even the mechanical

¹ Lepierre, *loc. cit.* Compare also Schwarz, *loc. cit.*

² Rubner, *Leyden's Handb. der Ernährungs-Therapie*, Vol. I. p. 114. Hammarsten, *Lehrb. der physiol. Chemie*, 1895, p. 247, etc.

conditions which obtain in the living stomach, which effect both a continuous churning of the food with the digestive juice, and a continuous removal of the products formed. Further, from the purely biological standpoint, according to the interesting experiments of Pawlow¹, the food seems by virtue of its relative digestibility to have an influence upon the nature of the secretion produced by the stomach to digest it. In addition, a possible stimulant action on the part of formaldehyde must not be overlooked, so far as concerns the secretion of the enzymes and their quality, apart from a possible stimulant action upon their activity. That formic aldehyde seems to have a stimulating action upon certain enzymes may be concluded from the experiments of Macfadyen, Morris and Roland² on Buchner's zymase, from those of Weigel and Merkel³ upon diastase, and from those of Kastle and Loewenhardt upon "lipase".

With regard to the experiments on animals, only those interest us in which the formic aldehyde was given by the mouth; even in these the formic aldehyde was in no case given with food, but in a free state in water, and when any effect was produced at all the drug was present in a concentration and in an amount far in excess of that in which it would be given as a preservative in food. So far as concerns the effect of formic aldehyde when given to animals admixed with food, for long periods, the results are conflicting, but, on the whole, seem to show that formic aldehyde in moderate doses has little or no influence upon the growth, weight, and general health of even young animals.

It will be seen from the above criticism that although we are provided with a copious indirect literature, no conclusions, in the absence of direct observations upon man, can be drawn concerning the possible effect upon him of the addition of small doses of formic aldehyde to his food. It was clear to us that the only way to fill this gap in the literature was to make such observations, and the kind of observation best calculated to give exact and definite data for conclusions was in our opinion a series of metabolic experiments.

¹ Pawlow, *Die Arbeit der Verdauungsdrüsen*, 1898.

² *Berichte der deutsch. chem. Gesellsch.*, 1900, p. 2782.

³ *Forschungsberichte über Lebensmittel*, etc., 1895, p. 91.

⁴ *loc. cit.*

Methods.

Our experiments were conducted upon the same three children (A, B and C) who served for our research "On the Influence of Boric Acid and Borax upon the General Metabolism of Children" (*Journ. of Hygiene*, vol. I. p. 168), and the methods employed (*loc. cit.* pp. 172—175 etc.) were identical.

The following table shows the percentage composition of the foods used:—

TABLE I.
SHOWING THE PERCENTAGE COMPOSITION OF FOODS.

—			Specific gravity	Water %	Fat %	Total carbohydrates %	Nitrogen %	Phosphoric acid %	Ash %
						Lactose			
Milk	I	...	1.0329	86.55	4.50	4.92	0.49	0.29	0.69
"	II	...	1.0331	86.92	3.85	4.03	0.58	0.28	0.70
"	III	...	1.0320	87.36	3.50	5.21	0.49	0.24	0.69
"	IV	...	1.0329	87.76	3.37	4.81	0.51	0.23	0.64
						Dextrose			
Bread	I	...	—	36.10	0.51	47.47	1.48	0.36	—
"	II	...	—	40.13	0.11	39.21	1.50	0.18	—
"	III	...	—	35.77	0.24	55.48	1.32	0.19	—
"	IV	...	—	29.83	0.23	59.13	1.78	0.27	0.89
"	V	...	—	34.42	0.11	60.80	1.55	0.19	0.89
						Lactose			
Butter	I	...	—	12.46	86.44	0.36	0.02	—	0.65
"	II	...	—	12.82	85.44	0.21	0.13	—	0.72
"	III	...	—	12.89	85.31	0.40	0.12	—	0.61
"	IV	...	—	12.68	86.00	0.14	0.11	—	0.50
Meat	I	...	—	75.00	5.38	—	2.95	0.41	—
"	Veal II	...	—	74.13	0.78	—	3.79	0.55	—
"	III	...	—	72.12	6.31	—	3.08	0.51	—
"	IV	...	—	72.60	6.67	—	3.23	0.53	1.00
"	V	...	—	69.04	7.12	—	3.52	0.46	1.07
"	VI	...	—	74.48	4.62	—	3.74	0.46	1.00
						Dextrose			
Apple	I	...	—	70.08	—	25.20	0.04	0.04	0.35
"	Compote II	...	—	56.66	—	37.66	0.05	0.05	0.38
"	" III	...	—	69.86	—	30.25	0.05	0.04	0.33
						Dextrose			
Toffee	—	1.06	2.20	18.24	0.03	—	—

OBSERVATION I. CHILD A.

The child was a healthy boy aged $2\frac{1}{2}$ years, weighed 14·6 kilos, and remained in good health throughout the whole observation. He consumed daily as follows, 200 g. of bread, 550 c.c. of milk, 20 g. of butter, 30 g. of meat, 50 g. of apple compote, 10 g. of sugar, 50 c.c. of water, 5 g. of toffee. This diet was very well taken and adhered to throughout the experiment. The whole observation extended over 28 days, seven days being taken for a fore period, and seven days for an after period; during the intermediate 14 days formic aldehyde was administered. In the first seven days of this intermediate period formic aldehyde was given in the morning and evening milk in such quantity that it was present in the proportion of 1 in 10,000: the actual quantity given per day was 0·05 g. formic aldehyde in 500 c.c. of milk. The formic aldehyde was added to the milk in the morning about 7.30 a.m.: 250 c.c. of the milk were taken at about 8.30 a.m., and 250 c.c. at 5 p.m.: it will be seen therefore that we not only gave freshly formalised milk, but milk which had been exposed to the action of formic aldehyde for a considerable time. The whole food per diem weighed approximately 900 g., it therefore follows that the total food and drink during the first week of the formic aldehyde period was formalised to the extent of 1 in 18,000. During the second formic aldehyde period the dose of formic aldehyde was doubled, viz. 0·10 g. per diem, equal to 1 in 5,000 in milk, and 1 in 9,000 in total food and drink. The increased dose, viz. 0·05 g., was occasionally given with the meal at dinner and occasionally in the milk. The analytical results obtained throughout the observation are recorded in the following table:

TABLE II.

SHOWING THE INFLUENCE OF FORMIC ALDEHYDE

PERIOD	—	Date	Dose	URINE						
				Quantity	Reaction	Specific gravity	Total sulphuric acid g	Ethereal sulphuric acid g	Uric acid g	Nitrogen g
FORE PERIOD		2 IV		375	Acid	1·0220	0·8925	0·0533	0·1823	5·07
		3 "		375	"	1·0235	0·8925	0·0533	0·1823	5·35
		4 "		410	Amphoteric	1·0200	1·0016	0·0538	0·2337	5·22
		5 "		340	Acid	1·0250	0·8305	0·0446	0·1938	5·55
		6 "		330	"	1·0230	0·8061	0·0433	0·1831	5·13
		7 "		375	Amphoteric	1·0225	0·9160	0·0492	0·2137	5·05
		8 "		320	Acid	1·0242	0·8841	0·0533	0·2160	5·60
	Total	7 days		2,525			6·2233	0·3508	1·4099	36·97
	Average	1 day		360		1·0229	0·8890	0·0501	0·2014	5·28
FIRST FORMIC ALDEHYDE PERIOD		9 IV	0·05	270	Acid	1·0269	0·7460	0·0449	0·1822	4·82
		10 "	0·05	315	"	1·0260	0·8703	0·0524	0·2126	5·30
		11 "	0·05	320	"	1·0259	0·8841	0·0532	0·2160	5·42
		12 "	0·05	320	"	1·0260	0·8841	0·0532	0·2160	5·43
		13 "	0·05	405	"	1·0179	0·8925	0·0550	0·2157	4·75
		14 "	0·05	320	"	1·0270	0·7052	0·0435	0·1704	5·55
		15 "	0·05	270	"	1·0289	0·5950	0·0367	0·1438	4·80
	Total	7 days	0·35	2,220			5·5777	0·3380	1·3567	36·07
	Average	1 day	0·05	317		1·0255	0·7968	0·0484	0·1938	5·15
SECOND FORMIC ALDEHYDE PERIOD		16 IV	0·1	325	Acid	1·0260	0·7162	0·0441	0·1731	5·19
		17 "	0·1	445	"	1·0175	0·9860	0·0604	0·2370	4·96
		18 "	0·1	315	"	1·0235	0·9131	0·0494	0·2126	5·19
		19 "	0·1	300	"	1·0240	0·8697	0·0470	0·2025	5·01
		20 "	0·1	310	"	1·0262	0·8986	0·0487	0·2092	5·08
		21 "	0·1	270	"	1·0266	0·7827	0·0423	0·1822	5·33
		22 "	0·1	295	"	1·0293	0·8552	0·0463	0·1991	5·83
	Total	7 days	0·7	2,260			6·0215	0·3382	1·4157	36·59
	Average	1 day	0·1	323		1·0247	0·8602	0·0483	0·2022	5·22
AFTER PERIOD		23 IV		355	Acid	1·0269	0·8516	0·0494	0·1679	5·47
		24 "		295	"	1·0272	0·7077	0·0410	0·1395	5·09
		25 "		370	"	1·0215	0·8875	0·0514	0·1750	4·89
		26 "		370	Amphoteric	1·0230	0·8875	0·0514	0·1750	5·28
		27 "		350	Acid	1·0200	0·8396	0·0487	0·1655	4·47
		28 "		415	Amphoteric	1·0200	0·9396	0·0624	0·2428	6·01
		29 "		345	"	1·0200	0·7811	0·0419	0·2018	4·86
	Total	7 days		2,500			5·8946	0·3562	1·2675	36·07
	Average	1 day		360		1·0227	0·8421	0·0509	0·1810	5·15

TABLE II.

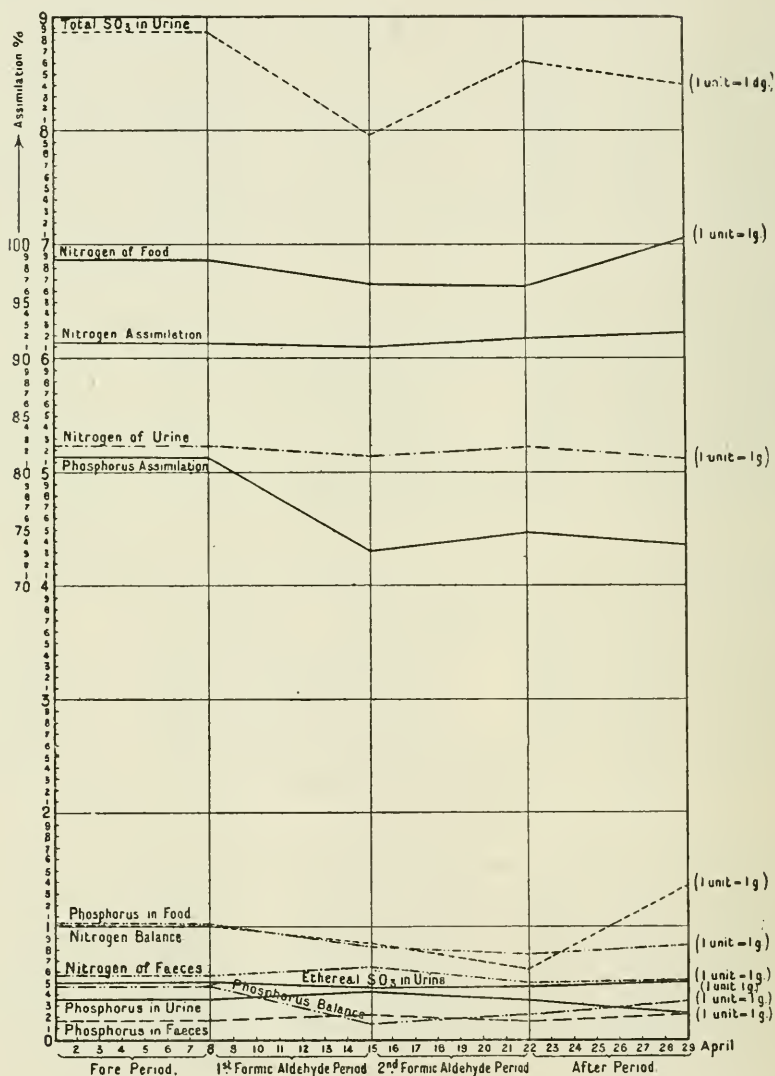
UPON THE GENERAL METABOLISM OF CHILD A.

FAECES				Nitrogen of food	Balance	Body weight	PHOSPHORUS				FAT		
Moist	Dry	Water %	Nitro- gen				Urine	Faeces	Food	Balance	Faeces	Food	Balance
g	g		g	g	g	kg	g	g	g	g	g	g	g
65	9.0	86.2	0.56	6.56	+0.93	14.61	0.4436	0.1809	1.10	+0.48	2.40	44.77	+42.37
49	9.2	81.2	0.58	6.81	+0.88		0.4436	0.1809	1.16	+0.54	2.45	47.02	+44.57
64	12.5	85.9	0.78	6.81	+0.81		0.3805	0.2440	1.18	+0.55	3.33	47.02	+43.69
—	—	—	—	6.85	+1.30		0.3155	—	1.02	+0.70	—	44.84	+44.84
68	14.5	78.7	0.84	6.60	+0.73		0.3062	0.2779	0.96	+0.44	3.81	38.82	+35.01
60	11.5	80.8	0.66	7.41	+1.70		0.3480	0.2255	0.96	+0.39	3.02	40.74	+37.72
59	12.4	77.3	0.72	7.16	+0.84	14.39	0.4838	0.2365	0.96	+0.24	3.26	40.74	+37.48
365	69.1		4.14	48.20	+7.19	-220g.	2.7212	1.3457	7.34	+3.34	18.27	303.95	+285.68
52	96	81.7	0.59	6.88	+1.02	Loss	0.3887	0.1922	1.05	+0.48	2.61	43.42	+40.81
51	11.4	77.7	0.68	7.16	+1.66	14.39	0.4082	0.2463	0.90	+0.25	2.97	40.48	+37.51
64	11.0	82.8	0.65	7.16	+1.21		0.4762	0.2376	0.90	+0.19	2.86	40.74	+37.88
41	8.4	79.6	0.49	6.79	+0.88		0.4838	0.1816	0.90	+0.23	2.19	40.74	+38.55
88	15.0	82.9	0.90	6.53	+0.20		0.4838	0.3242	0.79	-0.02	3.91	38.79	+34.88
—	—	—	—	6.32	+1.57		0.4957	—	0.79	+0.29	—	38.79	+38.79
76	15.2	80.0	0.88	6.33	-0.10		0.3917	0.3085	0.79	+0.09	3.76	38.79	+35.03
115	15.5	86.5	0.89	6.33	+0.64	14.61	0.3304	0.3147	0.79	+0.14	3.83	38.79	+34.96
435	76.5		4.49	46.62	+6.06	+220g.	3.0698	1.6129	5.86	+1.17	19.52	277.12	+257.60
62	10.9	82.6	0.64	6.66	+0.87	Gain	0.4385	0.2304	0.84	+0.17	2.79	39.59	+36.80
—	—	—	—	6.33	+1.14	14.61	0.3978	—	0.79	+0.39	—	38.79	+38.79
—	—	—	—	6.38	+1.42		0.5447	—	0.79	+0.25	—	38.90	+38.90
82	20.0	75.6	1.17	6.38	+0.02		0.3238	0.4615	0.79	+0.00	5.14	38.90	+33.76
55	11.2	79.6	0.66	6.38	+0.71		0.3084	0.2585	0.79	+0.22	2.87	38.90	+36.03
70	13.7	80.4	0.80	6.38	+0.50		0.3186	0.3162	0.79	+0.16	3.52	38.90	+35.38
—	—	—	—	6.38	+1.05		0.2773	—	0.79	+0.51	—	38.90	+38.90
65	15.5	76.2	0.91	6.39	-0.35	14.72	0.3033	0.3577	0.78	+0.12	3.98	39.04	+35.06
272	60.4		3.54	44.62	+4.49	+110g.	2.4739	1.3939	5.52	+1.65	15.51	272.33	+256.82
39	8.6	77.9	0.51	6.37	+0.64	Gain	0.3534	0.1991	0.79	+0.23	2.22	38.90	+36.69
57	14.8	74.0	0.87	7.31	+0.97	14.72	0.1974	0.3326	0.86	+0.33	4.08	39.02	+34.94
54	10.8	80.0	0.63	7.31	+1.59		0.1640	0.2427	0.86	+0.45	2.98	39.02	+36.04
61	13.2	78.4	0.77	7.39	+1.73		0.2057	0.2966	0.86	+0.36	3.64	38.31	+34.67
48	12.0	75.0	0.67	6.92	+0.97		0.2057	0.2882	0.78	+0.29	3.04	37.32	+34.28
—	—	—	—	6.92	+2.45		0.1946	—	0.78	+0.59	—	37.46	+37.46
—	—	—	—	6.92	+0.91		0.2922	—	0.78	+0.49	—	37.46	+37.46
106	14.9	85.9	0.84	6.92	+1.22	15.00	0.2429	0.3579	0.78	+0.18	3.78	37.46	+33.68
326	65.7		3.78	49.69	+9.84	+280g.	1.5025	1.5180	5.70	+2.69	17.52	266.05	+248.53
47	9.4	80.0	0.54	7.09	+1.40	Gain	0.2146	0.2168	0.81	+0.38	2.70	38.01	+35.50

The results expressed in the above table are graphically represented in the following curves:

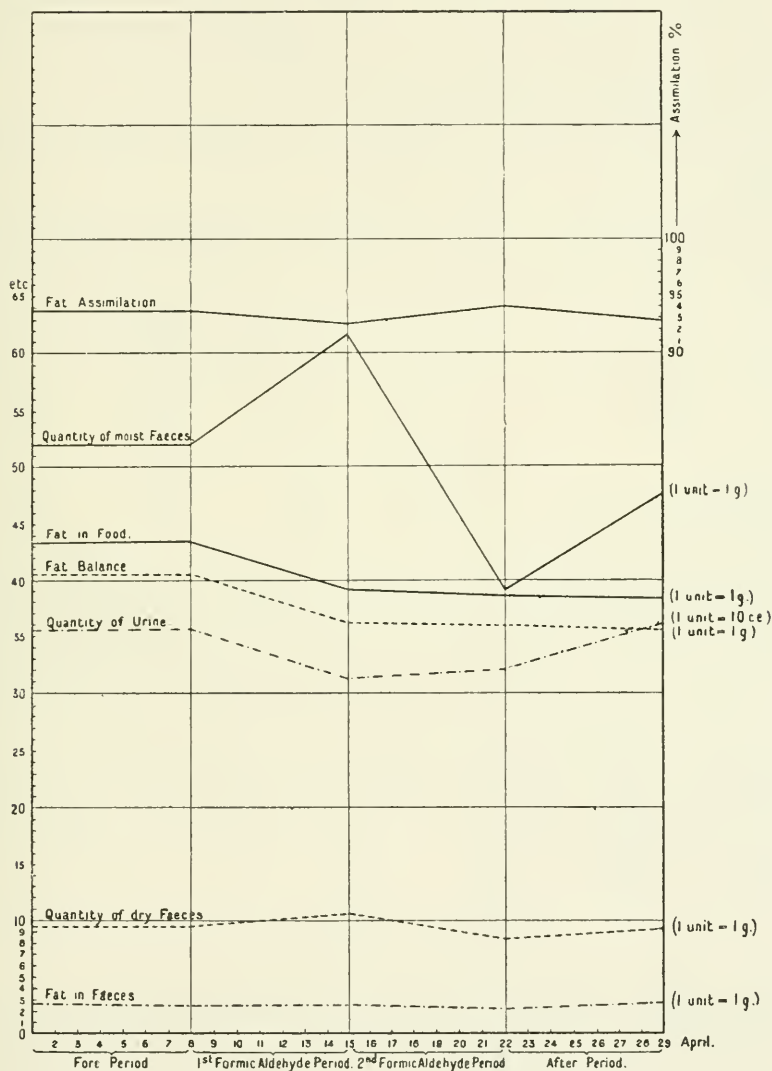
CURVE I.,

showing the influence of formic aldehyde upon nitrogen and phosphorus metabolism, etc.



CURVE II.,

showing the influence of formic aldehyde upon fat assimilation and quantity of faeces and urine.



Referring to the tables and curves relating to the *first observation*, we will classify our remarks under the following headings :

Nitrogen Metabolism.

In the fore period the daily quantity of nitrogen taken in the food was 6.88 g., of which 0.59 g. was not assimilated, being lost with the faeces, corresponding to 8.58 %. The assimilation of nitrogen in the fore period amounted therefore to 91.42 %.

With the urine 5.28 g. of nitrogen were excreted, and if this be subtracted from the amount assimilated we obtain a daily balance of +1.02.

To avoid repetition we give the results with regard to the nitrogen balance and assimilation during the different periods in tabular form :

—	Fore period	First F.A. period	Second F.A. period	After period
Nitrogen of Food	6.88	6.66	6.37	7.09
„ „ Urine	5.28]	5.15]	5.22]	5.15]
„ „ Faeces	0.59]	0.64]	0.51]	0.54]
Balance	+1.02	+0.87	+0.64	+1.40
Assimilation %	91.42	91.22	91.99	92.38
Nitrogen % in dry Faeces ...	6.1	5.9	5.9	5.8

From these results we are justified in drawing the conclusion that the formic aldehyde had no appreciable influence upon proteid metabolism in the case of this child. If with the necessary restrictions we regard the nitrogen excreted in the faeces as an index of the digestibility of the food, we find that in this case the addition of formic aldehyde to the proteid constituents of the food has been without influence in this direction. This is best seen from the average percentage of nitrogen in the faeces in the several periods. The assimilation and the balance are likewise not affected.

Phosphorus Metabolism.

The daily average of phosphorus in the food in the fore period was 1.05 g., of which 0.1922 were lost, being excreted in the faeces. Phosphorus was therefore assimilated to the extent of 81.70 %. The relative excretion, etc. of phosphorus in the four periods we give in tabular form :

—	Fore period	First F.A. period	Second F.A. period	After period
Phosphorus of Food	1·05	0·84	0·79	0·81
„ „ Urine	0·3887	0·3885	0·3534	0·2146
„ „ Faeces	0·1922	0·2304	0·1991	0·2168
Balance	+0·48	+0·17	+0·23	+0·38
Assimilation %	81·70	72·57	74·80	73·24
Phosphorus % in dry Faeces ...	2·0	2·1	2·3	2·3

From these figures it will be seen that the absolute quantity of phosphorus in the urine in the first formic aldehyde period is slightly increased (0·5 g.), in the second formic aldehyde period it falls, however, slightly below the fore period value and in the after period still further. As at the same time the assimilation of phosphorus as measured by the phosphorus excreted in the faeces is somewhat lessened, it would seem that during the first period formic aldehyde tended to stimulate phosphorus metabolism. The absolute changes are however so small that they can only be regarded as indicating what might be the possible effect of formic aldehyde in larger doses.

Fat Assimilation.

The daily average of fat in the food during the fore period was 43·42 g., of this 2·61 was lost, being excreted with the faeces, leaving a balance of +40·81 g. The assimilation therefore amounted to 93·99 %. These results and those of the following periods are recorded in tabular form :

—	Fore period	First F.A. period	Second F.A. period	After period
Fat in Food	43·42	39·59	38·90	38·01
„ Faeces	2·61	2·79	2·22	2·70
Fat balance	+40·81	+36·80	+36·69	+35·50
Assimilation %	93·99	92·96	94·30	92·90
Fat % in dry Faeces	27·1	25·6	25·8	28·7

From these figures it will be seen that the quantity of fat in the faeces during the formic aldehyde periods did not increase, if anything decreased, in proportion to the faeces. The fat assimilation and fat balance remained unaffected.

In connection with the fat in the faeces, we estimated the *lecithin*

according to the method described above. The results expressed as a percentage of total fat are as follows :

—	Fore period	First F.A. period	Second F.A. period	After period
Lecithin in grammes of 100 g. fat	15.08	11.31	13.16	4.28

As we enter into this subject later, we would only point out here that formic aldehyde seemed to have an influence in diminishing the excretion of lecithin by the faeces, and that this influence extended into the after period.

Having considered the most important factors in metabolism as investigated by us and drawn the conclusion that formic aldehyde had little if any influence upon them, we turn our attention to certain other factors which although of minor importance ought not to be overlooked.

On referring to the chief table (Table II)¹ it will be seen that the total *quantity of urine* in the first formic aldehyde period decreased, whilst the quantity of faeces and especially their percentage of water increased. In the second formic aldehyde period the quantity of urine increased slightly, whilst the quantity of faeces and their water percentage decreased. It would thus seem that formic aldehyde had a tendency to produce a retention of water by the body. A rise in the specific gravity of the urine occurred *pari passu* with its diminution of volume.

If we regard the *uric acid* figures during this observation we shall see that the average excretion of uric acid during the first formic aldehyde period underwent a very slight diminution along with the total nitrogen, and this would justify us in concluding that the urea and ammonia varied in the same direction. The analytical figures so far as concerns the average total *sulphuric acid* excretion show that formic aldehyde exerted upon it a barely appreciable effect, suggesting in connection with the nitrogen figures above, if we draw conclusions from them at all, that formic aldehyde exerted a slightly inhibitory action upon the breaking up of proteids in the body. Even these conclusions can only be applied to the first formic aldehyde period taken as a whole.

¹ In these remarks throughout the entire paper we refer to the average daily excretion in question.

The strongly antiseptic properties of formic aldehyde render an inquiry as to its effect upon intestinal putrefaction, as measured by the quantity of ethereal sulphates in the urine, of interest. If we refer to the absolute figures in Table II we are forced to the conclusion that the slight diminution of ethereal sulphates which occurred during both formic aldehyde periods is not sufficient to indicate any inhibitory effect upon intestinal putrefaction¹. This seems to show that when formic aldehyde is taken with the food in these proportions, it does not in healthy children occur as such in the intestines.

So far as concerns body-weight, it will be observed that there was a slight increase in the two formic aldehyde periods, which seems however to be due not to an increased flesh formation or fat retention, but to a retention of water as indicated by the diminished excretion of water in the urine or faeces.

The results relevant to the observations made above are summarised in the following table:

TABLE II A.

—	Nitrogen assimilation, %	% N. of dry faeces	Phosphorus assimilation, %	% P. of dry faeces	Fat assimilation, %	% Fat of dry faeces	A* B	N † SO ₃
Fore period ...	91.42	6.1	81.70	2.0	93.99	27.1	16.7	6.1
First F.A. period	91.22	5.9	72.57	2.1	92.96	25.6	15.5	6.4
Second F.A. period	91.99	5.9	74.80	2.3	94.30	28.8	16.9	6.1
After period ...	92.38	5.8	73.24	2.3	92.90	28.7	15.5	6.1

* A = Inorganic SO₃.

B = Ethereal SO₃.

† $N = \frac{\text{Nitrogen of Urine}}{\text{SO}_3 \text{ of Urine}}$.

OBSERVATION II. CHILD B.

The child was a healthy boy aged 5 years, weighing 17.2 kilos, and remained in good health throughout the whole observation. He consumed daily 250 g. of bread, 600 c.c. of milk, 20 g. of butter, 50 g. of meat, 50 g. of apple compote, 10 g. of sugar, 50 c.c. of water, and 5 g. of toffee. The duration and arrangement of the observation was as in Child A. The formic aldehyde was administered in an identical manner and dose, but as in this case the total food was slightly increased, the proportion of formic aldehyde in it was slightly less. The analytical results obtained throughout this observation are recorded in the following table:

¹ The ratio of total sulphuric acid to actual sulphuric acid is likewise not affected; further, as has been pointed out by many observers, this ratio is of no special importance.

TABLE III.

SHOWING THE INFLUENCE OF FORMIC ALDEHYDE

PERIOD	————	Date	Dose	URINE						
				Quantity	Reaction	Specific gravity	Total sulphuric acid g	Ethereal sulphuric acid g	Uric acid g	Nitrogen g
			g	c.c.						
FORE PERIOD		2 IV.		340	Acid	1·0220	0·7782	0·0623	0·1122	4·58
		3 "		360	"	1·0195	0·8240	0·0659	0·1188	4·92
		4 "		390	"	1·0225	1·0600	0·0819	0·1346	6·03
		5 "		150	"	1·0290	0·4076	0·0315	0·0518	2·61
		6 "		540	"	1·0260	1·4674	0·1135	0·1863	9·76
		7 "		360	"	1·0260	0·9783	0·0756	0·1242	6·09
		8 "		390	"	1·0249	1·1890	0·0750	0·1082	6·82
	Total	7 days		2,530			6·7045	0·5057	0·8361	40·81
	Average	1 day		361		1·0245	0·9578	0·0772	0·1194	5·83
FIRST FORMIC ALDEHYDE PERIOD		9 IV.	0·05	300	Acid	1·0295	0·9146	0·0577	0·0833	6·02
		10 "	0·05	325	"	1·0300	0·9908	0·0625	0·0902	6·82
		11 "	0·05	335	"	1·0280	1·0210	0·0644	0·0929	6·73
		12 "	0·05	365	"	1·0240	1·1130	0·0702	0·1013	5·63
		13 "	0·05	345	"	1·0252	0·9736	0·0627	0·0647	5·78
		14 "	0·05	270	"	1·0300	0·7619	0·0490	0·0506	5·27
		15 "	0·05	335	"	1·0305	0·9453	0·0608	0·0628	6·55
	Total	7 days	0·35	2,275			6·7202	0·4273	0·5458	42·80
	Average	1 day	0·05	325		1·0282	0·9600	0·0610	0·0779	6·12
SECOND FORMIC ALDEHYDE PERIOD		16 IV.	0·1	345	Acid	1·0220	0·9736	0·0627	0·0647	5·17
		17 "	0·1	410	"	1·0215	1·1570	0·0745	0·0769	5·82
		18 "	0·1	395	"	1·0226	1·1755	0·0729	0·1185	6·01
		19 "	0·1	380	"	1·0245	1·1131	0·0702	0·1140	6·73
		20 "	0·1	280	"	1·0280	0·8332	0·0517	0·0840	5·35
		21 "	0·1	380	"	1·0255	1·1131	0·0702	0·1140	6·29
		22 "	0·1	275	"	1·0293	0·8183	0·0508	0·0825	5·59
	Total	7 days	0·7	263			7·1838	0·4530	0·6546	40·96
	Average	1 day	0·1	323		1·0247	1·0262	0·0647	0·0935	5·85
AFTER PERIOD		23 IV.		395	Acid	1·0276	0·8998	0·0536	0·0593	6·18
		24 "		495	"	1·0180	1·1276	0·0672	0·0743	5·49
		25 "		410	"	1·0275	0·9337	0·0557	0·0615	7·39
		26 "		535	"	1·0190	1·2184	0·0726	0·0803	6·41
		27 "		480	"	1·0210	1·0934	0·0652	0·0720	6·55
		28 "		560	"	1·0190	1·2710	0·0795	0·0420	7·38
		29 "		455	"	1·0200	1·0330	0·0646	0·0341	6·62
	Total	7 days		3,330			7·5769	0·4584	0·4235	46·02
	Average	1 day		476		1·0216	1·0824	0·0655	0·0605	6·57

TABLE III.

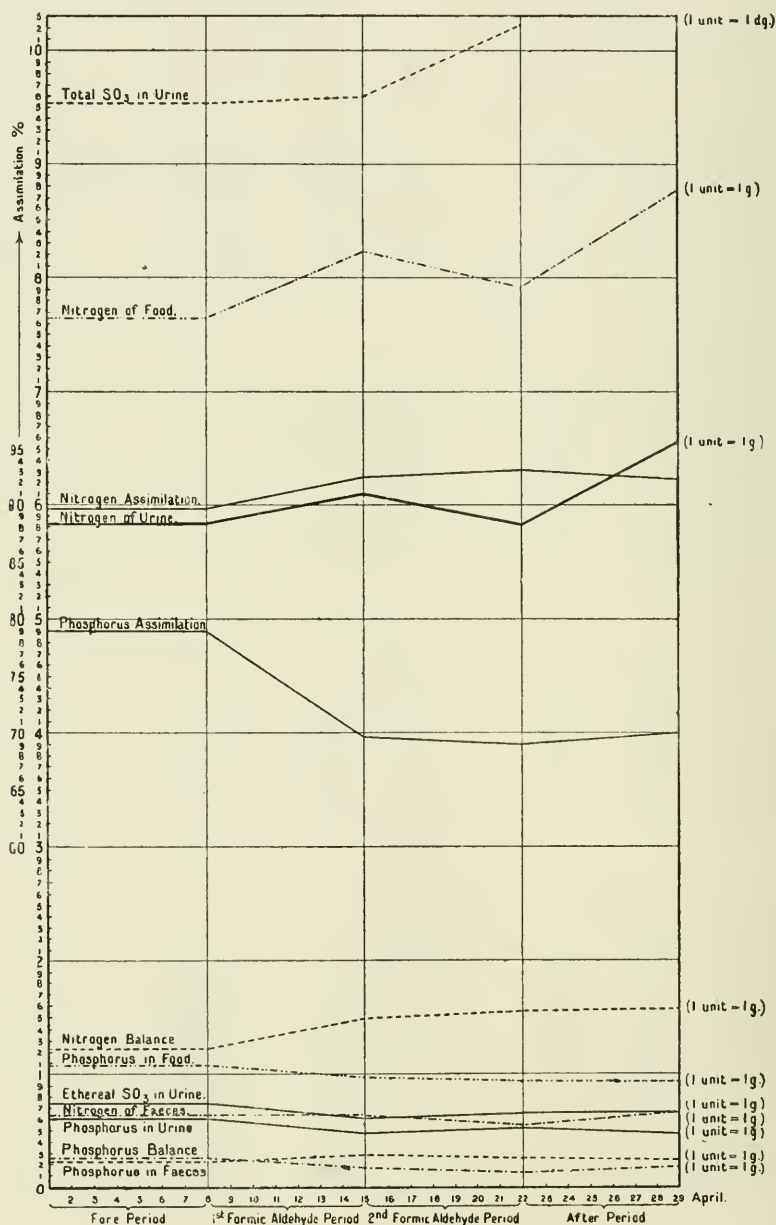
UPON THE GENERAL METABOLISM OF CHILD B.

FAECES				Nitro- gen of food	Balance	Body weight	PHOSPHORUS				FAT		
Moist	Dry	Water %	Nitro- gen				Urine	Faeces	Food	Balance	Faeces	Food	Balance
g	g		g	g	g	kg	g	g	g	g	g	g	g
85	19.5	77.1	1.24	7.15	+1.33	17.21	0.8092	0.3918	1.14	-0.06	4.87	45.85	+40.98
7	2.2	68.6	0.14	7.40	+2.34		0.8568	0.0454	1.20	+0.30	0.56	48.10	+47.54
56	13.0	76.8	0.85	7.40	+0.52		0.5413	0.2681	1.20	+0.39	3.33	48.10	+44.77
112	21.5	80.8	1.24	7.44	+3.59		0.2082	0.5215	1.07	+0.34	5.59	45.00	+39.41
—	—	—	—	7.19	-2.57		0.7495	—	1.01	+0.26	—	38.98	+38.98
57	15.4	73.0	0.89	8.01	+1.03		0.4994	0.3736	1.01	+0.14	4.01	40.90	+36.89
—	—	—	—	9.17	+2.35	17.27	0.5974	—	1.05	+0.45	—	40.96	+40.96
317	71.6		4.36	53.76	+8.59	Total	4.2618	1.6004	7.68	+1.82	18.36	307.89	+289.53
45	10.2	77.4	0.62	7.68	+1.23	Gain +60g.	0.6088	0.2286	1.09	+0.26	2.62	43.98	+41.36
9	3.0	66.7	0.22	8.82	+2.58	17.27	0.4596	0.0798	1.04	+0.50	0.71	43.72	+43.01
39	8.0	79.5	0.57	8.82	+1.43		0.4979	0.2128	1.04	+0.33	1.90	43.72	+41.82
126	31.7	74.8	2.28	8.37	-0.64		0.5132	0.8432	1.04	-0.32	7.54	44.04	+36.50
—	—	—	—	8.06	+2.43		0.5592	—	0.92	+0.36	—	41.92	+41.92
81	17.5	78.4	0.58	7.84	+1.48		0.4982	0.4449	0.92	-0.02	4.16	41.92	+37.76
—	—	—	—	7.85	+2.58		0.3899	—	0.92	+0.53	—	41.92	+41.92
75	20.0	73.3	0.67	7.85	+0.63		0.4838	0.5085	0.92	-0.07	4.75	41.92	+37.17
330	80.2		4.32	57.61	+10.49		3.4018	2.0892	6.80	+1.32	19.06	299.16	+280.07
47	11.5	75.7	0.62	8.23	+1.49		0.4859	0.2984	0.97	+0.19	2.72	42.73	+40.01
—	—	—	—	7.85	+2.68		0.4982	—	0.92	+0.42	—	41.92	+41.92
100	24.5	75.5	0.80	7.95	+1.33		0.5920	0.6229	0.92	-0.29	5.82	42.10	+36.28
—	—	—	—	7.95	+1.94		0.5799	—	0.92	+0.34	—	42.10	+42.10
54	9.0	83.3	0.53	7.95	+0.69		0.5579	0.2384	0.92	+0.12	2.25	42.10	+39.85
85	21.6	74.6	1.26	7.95	+1.34		0.4110	0.5722	0.92	-0.06	5.41	42.10	+36.69
19	5.3	72.1	0.31	7.95	+1.35		0.5379	0.1404	0.92	+0.22	1.33	42.10	+40.77
57	15.7	72.5	0.92	8.06	+1.55	17.49	0.4037	0.4159	0.91	+0.09	3.93	42.33	+38.40
315	76.1		3.82	55.66	+10.88	Total	3.6006	1.9898	6.43	+0.84	18.74	294.65	+276.01
45	10.8	75.8	0.54	7.95	+1.56	Gain +270	0.5143	0.2842	0.92	+0.12	2.68	42.09	+39.43
50	11.6	76.8	0.69	8.76	+1.89	17.49	0.4298	0.2679	1.01	+0.31	2.53	42.31	+39.78
—	—	—	—	9.21	+3.72		0.5386	—	1.01	+0.47	—	42.31	+42.31
88	21.8	75.0	1.31	9.31	+0.61		0.4461	0.5034	1.01	+0.06	4.75	41.53	+36.78
—	—	—	—	8.59	+2.18		0.5820	—	0.91	+0.33	—	39.98	+39.98
51	12.4	75.7	0.75	8.59	+1.29		0.5222	0.3289	0.91	+0.06	2.77	40.12	+37.35
61	14.9	75.6	0.89	8.59	+0.32		0.4816	0.3952	0.91	+0.03	3.32	40.12	+36.80
87	16.2	81.4	0.97	8.59	+1.00	17.49	0.3913	0.4296	0.91	+0.09	3.62	40.12	+36.50
337	76.9		4.61	61.64	+11.01	Gain	3.3916	1.9250	6.67	+1.35	16.99	306.49	+269.50
48	10.9	77.2	0.66	8.80	+1.57	≈ 0	0.4845	0.2750	0.95	+0.19	2.42	43.78	+38.50

The results expressed in the above table are graphically represented in the following curves:

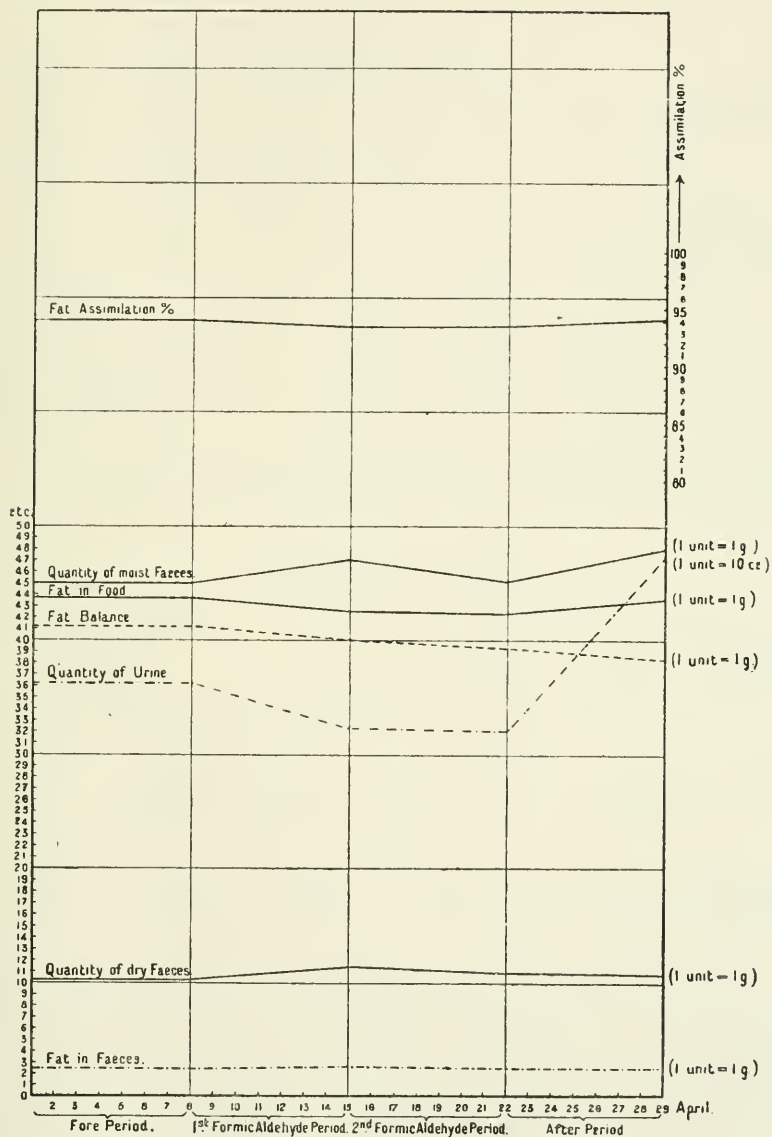
CURVE III.,

showing the influence of formic aldehyde upon nitrogen and phosphorus metabolism, etc.



CURVE IV.,

showing the influence of formic aldehyde upon fat assimilation and the quantity of faeces and urine.



Nitrogen Metabolism.

Adopting the same method of reasoning as in Observation I. we arrive at the following results with regard to the nitrogen balance and assimilation. These are best expressed in a tabular form.

—	Fore period	First F.A. period	Second F.A. period	After period
Nitrogen of Food	7.68	8.23	7.95	8.80
„ „ Urine	5.83 }	6.12 }	5.85 }	6.57 }
„ „ Faeces	0.62 }	0.62 }	0.54 }	0.66 }
Balance	+1.23	+1.49	+1.56	+1.57
Assimilation %	89.82	92.46	93.21	92.50
Nitrogen % in dry Faeces ...	6.0	5.4	5.0	6.0

From these results we can draw the conclusion that formic aldehyde in both periods seemed to have a slightly beneficial effect upon proteid assimilation. This is indicated by a rise in the percentage of nitrogenous food assimilated. The nitrogen percentage of the faeces shows a diminution. This result may find an explanation in a stimulating action of formic aldehyde upon the secretion of the digestive enzymes.

Phosphorus Metabolism.

The results in this connection expressed in a tabular form are as follows:

—	Fore period	First F.A. period	Second F.A. period	After period
Phosphorus of Food	1.09	0.97	0.92	0.95
„ „ Urine	0.6088 }	0.4859 }	0.5413 }	0.4845 }
„ „ Faeces	0.2286 }	0.2984 }	0.2842 }	0.2750 }
Balance	+0.26	+0.19	+0.12	+0.19
Assimilation %	79.03	69.23	69.11	71.05
Phosphorus in dry Faeces % ...	2.2	2.6	2.6	2.5

From the above table it will be seen that the absolute differences in the several periods are small. Nevertheless if any positive conclusion may be drawn, it is in this case that the formic aldehyde had a slightly depressing effect upon the assimilation of phosphorus as expressed by the slight increase of the phosphorus in the faeces. It must not be overlooked that the percentage figures in this case are a very large

magnification of the absolute variations in amount. The formic aldehyde seemed to have no influence in stimulating the breaking down of the phosphorus-containing substances in the body itself; in fact there seems to be a suggestion, from the diminished amount of phosphorus in the urine, that it acted in the opposite direction.

Fat Assimilation.

As in the preceding cases we express these results for the sake of convenience in the following tables:

—	Fore period	First F.A. period	Second F.A. period	After period
Fat in Food	43·98	42·73	42·09	43·78
„ Faeces	2·62	2·72	2·68	2·42
Balance	+ 41·36	+ 40·01	+ 39·43	+ 38·50
Assimilation %	94·05	93·63	93·63	94·47
Fat in dry Faeces %	25·7	23·7	24·8	22·1

From these figures it will be seen that the remarks we made with regard to the effect of formic aldehyde upon fat assimilation in Observation I. hold good in this case, viz. that there is no influence.

In so far as concerns lecithin the following figures express our results:

—	Fore period	First F.A. period	Second F.A. period	After period
Lecithin in grammes of 100 g. fat	21·09	22·60	14·59	17·30

Formic aldehyde seems to have the same influence here as in Observation I.

With regard to the remaining factors of general metabolism in this case it will be seen from the chief table (Table III) that the *quantity of urine* as in Observation I. decreased in the first formic aldehyde period, but that the influence upon the quantity of faeces and their water content is less marked. In the second formic aldehyde period the quantity of urine continued to be less than that of the fore period, whilst the *quantity of faeces* and their water content remained practically constant. In the after period the quantity of urine increased to an amount exceeding that of the fore period. The quantity of faeces

and their water content remained in this period also constant. It would seem therefore that, as in the former case, formic aldehyde had a tendency to cause a retention of water in the body. The reaction of the urine remained acid through the whole observation. The *uric acid* excretion diminished to some extent in the formic aldehyde period, while the total nitrogen and the total sulphuric acid remained practically unaffected. If the slightness of the changes do not preclude us from drawing any conclusions at all, we should infer that in this case formic aldehyde exerted a specific action upon either the formation or retention of uric acid. The result in this case is the more interesting in that this child seemed to belong to the uric acid type. Under the influence of formic aldehyde the *etheral sulphates* of the urine underwent a slight diminution; this was more pronounced than in Observation I. There seemed, however, to be practically no action upon intestinal putrefaction. We are the more entitled to draw this conclusion as the indoxyl reaction was equally intense throughout the whole observation. The *body weight* as in the former observation went up in the formic aldehyde period and remained constant in the after period. The increase in the formic aldehyde periods must be ascribed rather to a retention of water than to actual growth. We may epitomise the above observations in tabular form as follows:

TABLE III A.

—	Nitrogen assimilation %	% N. of dry faeces	Phosphorus assimilation %	% P. of dry faeces	Fat assimilation %	% fat of dry faeces	A * B	N † SO ₃
Fore period ...	91·42	6·1	81·00	2·0	93·99	27·1	16·7	6·1
First F.A. period	91·22	5·9	72·57	2·1	92·96	25·6	15·5	6·4
Second F.A. period	91·99	5·9	74·80	2·3	94·30	25·8	16·9	6·1
After period ...	92·38	5·8	73·24	2·3	92·90	28·7	15·5	6·1

* As in Table II A.

† As in Table II A.

OBSERVATION III. CHILD C.

This child was a delicate girl, aged four years; she was convalescent from pneumonia and was, as compared with the other children, ill-nourished, and poorly developed. Her weight was 15 kilos. She consumed daily 200 g. of bread, 550 c.c. of milk, 20 g. of butter, 30 g. of meat, 50 g. of apple compote, 10 g. of sugar, 50 c.c. of water, 5 g. of toffee. The observation lasted for 21 days; seven days were used as fore period, seven days as formic aldehyde period and seven days as after period. The method of administering the formic aldehyde was the same as in the previous observations, but it was given in this case throughout in a concentration of

1 in 5,000 of milk, occasionally some of the formic aldehyde was given in the meat; the total food was formalised to the extent of 1 in 9,000. The total quantity given per diem was 0.1 g. The child's general health and behaviour did not seem to be affected in any way throughout the whole observation. The analytical results obtained throughout this observation are recorded in the table on pp. 354, 355.

Nitrogen Metabolism.

Following the same methods as before, the results in this connection are expressed in tabular form as follows:

—	Fore period	F.A. period	After period
Nitrogen in Food per diem ...	6.65	7.01	6.99
" " Urine " ...	4.84	5.13	5.29
" " Faeces " ...	0.54	0.77	0.68
Balance	+1.27	+1.11	+1.02
Assimilation %	91.88	89.01	90.27
Nitrogen % in dry Faeces ...	6.4	5.07	6.2

From these figures, speaking generally, we cannot say that the nitrogenous metabolism was to any extent affected. The quantity of nitrogen in the faeces however was certainly increased, and the effect seemed to be prolonged into the after period. The difference in absolute quantity as compared to the fore period is however very small (0.23 g. in the formic aldehyde period, and 0.14 g. in the after period). If one were to regard this result superficially one would be tempted to at once draw the conclusion that formic aldehyde in this proportion, viz. 1 in 5,000, had rendered the proteid constituents of the food less digestible. Upon closer inspection, however, it seems that this increase of nitrogen in the faeces is rather to be explained by formic aldehyde exerting a slight irritant action upon the intestine, involving an increased shedding of epithelial cells. We are brought to this conclusion by the fact that the total quantity of the faeces was increased, and that the effect was prolonged into the after period. In this connection, however, we must not overlook the possibility of formic aldehyde exerting an inhibitory action upon the *secretion* of the digestive enzymes. As we shall discuss this later in another connection we shall say nothing further about it here.

TABLE IV.

SHOWING THE INFLUENCE OF FORMIC ALDEHYDE UPON

PERIOD	—	Date	Dose g	URINE						
				Quantity c.c.	Reaction	Specific gravity	Total sulphuric acid g	Ethereal sulphuric acid g	Uric acid g	Nitrogen g
FORE PERIOD		18 IV.		265	Acid	1·0260	0·8326	0·0684	0·1187	4·62
		19 "		245	"	1·0288	0·7618	0·0632	0·1097	4·76
		20 "		275	"	1·0287	0·8640	0·0709	0·1232	4·86
		21 "		280	"	1·0292	0·8798	0·0722	0·1253	4·72
		22 "		205	"	1·0316	0·6441	0·0529	0·1918	4·20
		23 "		330	"	1·0300	0·8038	0·0498	0·1795	5·83
		24 "		305	"	1·0250	0·7430	0·0461	0·1659	4·86
	Total	7 days		1,905			5·5291	0·4235	1·0141	33·85
	Average	1 day		258		1·0282	0·7898	0·0605	0·1306	4·84
FORMIC ALDEHYDE PERIOD		25 IV.	0·1	360	Amphoteric	1·0270	0·8769	0·0544	0·1958	5·64
		26 "	0·1	250	Acid	1·0190	0·6090	0·0378	0·1360	3·21
		27 "	0·1	420	Amphoteric	1·0180	1·0208	0·0634	0·2285	5·33
		28 "	0·1	335	"	1·0250	0·8348	0·0556	0·1715	5·84
		29 "	0·1	340	"	1·0230	0·8473	0·0564	0·1740	5·13
		30 "	0·1	260	"	1·0180	0·6479	0·0432	0·1331	2·89
		1 V.	0·1	415	Acid	1·0250	1·0340	0·0689	0·2124	7·88
	Total	7 days	0·7	2,380			5·8707	0·3797	1·2513	35·92
	Average	1 day	0·1	340		1·0221	0·8386	0·0542	0·1787	5·13
AFTER PERIOD		2 V.		300	Acid	1·0281	0·9141	0·0609	0·1008	5·24
		3 "		275	Amphoteric	1·0272	0·8379	0·0558	0·0924	4·92
		4 "		280	"	1·0286	0·8532	0·0568	0·0940	5·68
		5 "		270	"	1·0293	0·8227	0·0548	0·0907	5·01
		6 "		260	Acid	1·0293	0·7922	0·0528	0·0874	6·57
		7 "		210	Amphoteric	1·0276	0·7757	0·0379	0·0189	3·81
		8 "		255	"	1·0318	0·9227	0·0450	0·0229	5·80
	Total	7 days		1,850			5·9185	0·3640	0·5071	37·03
	Average	1 day		264		1·0288	0·8455	0·0520	0·0724	5·29

TABLE IV.

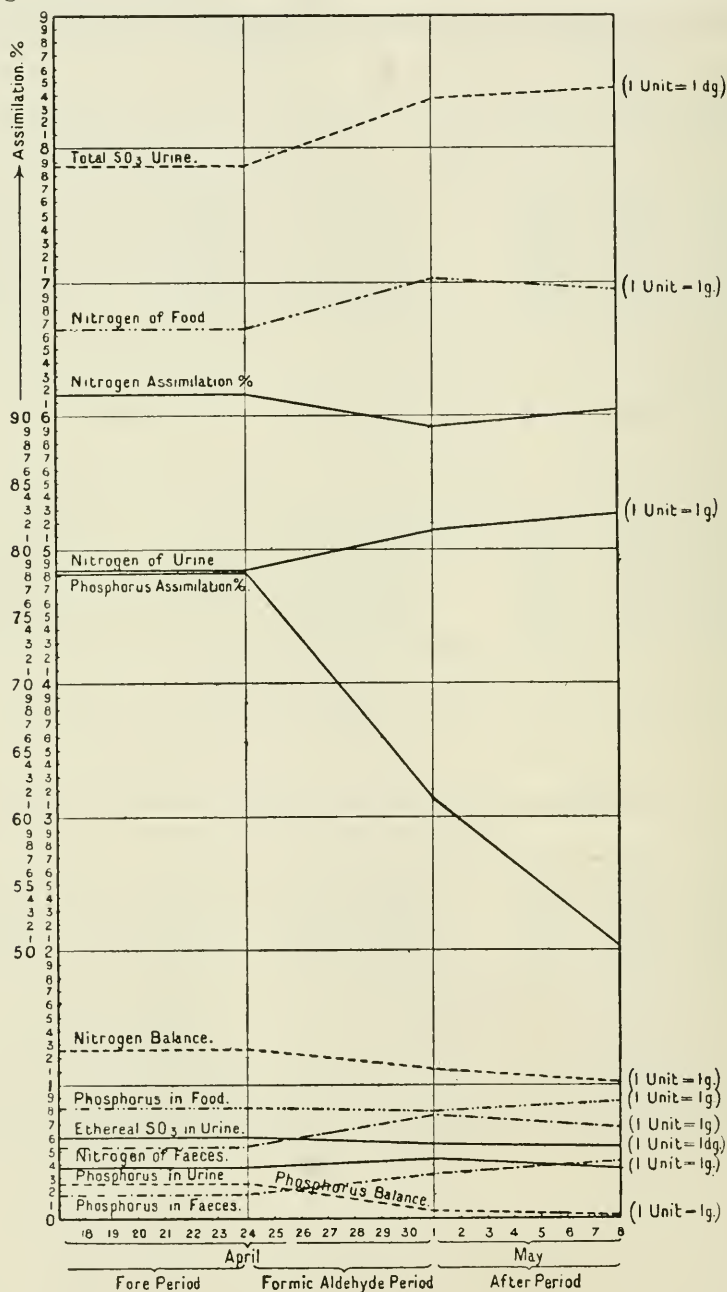
THE GENERAL METABOLISM OF THE INVALID CHILD C.

FAECES				Nitro- gen of food	Balance	Body weight	PHOSPHORUS				FAT		
Moist	Dry	Water %	Nitro- gen				Urine	Faeces	Food	Balance	Faeces	Food	Balance
g	g		g	g	g	kg	g	g	g	g	g	g	g
97	14.0	85.6	0.89	6.35	+0.84	15.06	0.4123	0.2915	0.79	+0.09	2.94	38.90	+35.96
—	—	—	—	6.35	+1.59	—	0.3813	—	0.79	+0.41	—	38.90	+38.90
63	12.5	80.2	0.80	6.35	+0.69	—	0.4279	0.2602	0.79	+0.10	2.63	38.90	+36.27
—	—	—	—	6.35	+1.63	—	0.4357	—	0.79	+0.35	—	38.90	+38.90
100	20.0	80.0	1.27	6.44	+0.97	—	0.3189	0.4164	0.78	+0.04	4.20	39.04	+34.84
75	12.7	83.1	0.82	7.36	+0.71	—	0.3683	0.2644	0.86	+0.23	2.67	39.02	+36.35
—	—	—	—	7.36	+2.50	15.12	0.3402	—	0.86	+0.52	—	39.02	+39.02
<hr/>													
335	59.2		3.78	46.56	+8.93	+60 g.	2.6846	1.2325	5.66	+1.74	12.44	272.68	+260.24
48	8.4	82.3	0.54	6.65	+1.27	Gain	0.3835	0.1760	0.81	+0.25	1.77	38.95	+37.18
<hr/>													
54	15.7	72.8	0.84	7.45	+0.97	15.12	0.4016	0.3532	0.86	+0.11	3.57	38.31	+34.74
77	15.0	80.5	0.81	6.89	+2.87	—	0.2790	0.3375	0.78	+0.16	3.41	37.32	+33.91
69	11.6	83.2	0.63	6.89	+0.93	—	0.4687	0.2610	0.78	+0.05	2.63	37.46	+34.83
37	10.3	72.2	0.56	6.89	+0.49	—	0.4559	0.2317	0.78	+0.09	2.34	37.46	+35.12
136	21.9	83.9	1.32	6.89	+0.44	—	0.4637	0.4861	0.78	-0.17	4.98	37.46	+32.48
35	7.5	78.6	0.45	7.05	+3.71	—	0.3546	0.1664	0.78	+0.26	1.71	37.56	+35.85
74	13.5	81.8	0.81	7.05	-1.64	15.40	0.5660	0.2996	0.78	-0.09	3.07	37.56	+34.49
<hr/>													
482	95.5		5.42	49.11	+7.77	+280 g.	2.9895	2.1355	5.54	+0.41	21.71	263.13	+241.42
69	13.6	80.3	0.77	7.01	+1.11	Gain	0.4270	0.3050	0.79	+0.06	3.10	37.59	+34.49
<hr/>													
21	4.4	79.0	0.30	7.08	+1.54	15.40	0.4119	0.5114	0.78	-0.14	0.89	37.56	+36.67
113	22.0	80.5	1.49	7.08	+0.67	—	0.3774	0.1023	0.78	+0.30	3.16	37.56	+34.40
28	3.6	87.1	0.21	7.06	+1.17	—	0.3843	0.9736	0.84	-0.52	0.68	36.20	+35.52
105	18.1	82.7	1.07	6.92	+0.85	—	0.3705	0.4895	0.84	-0.02	3.43	45.78	+42.35
47	8.5	81.9	0.51	6.92	-0.16	—	0.3568	0.2299	0.84	+0.25	1.61	45.78	+44.17
87	13.9	84.0	0.83	6.92	+2.28	—	0.3533	0.3759	0.84	+0.11	2.63	45.78	+43.15
31	6.6	78.7	0.39	6.97	+0.78	15.62	0.4289	0.1785	0.84	+0.23	1.25	45.78	+44.53
<hr/>													
432	77.1		4.80	48.95	+7.13	+220 g.	2.6831	2.8611	5.76	+0.22	13.65	294.44	+280.79
62	11.0	81.9	0.68	6.99	+1.02	Gain	0.3833	0.4087	0.82	+0.03	1.95	42.06	+40.11

The results expressed in the above table are graphically represented in the following curves:

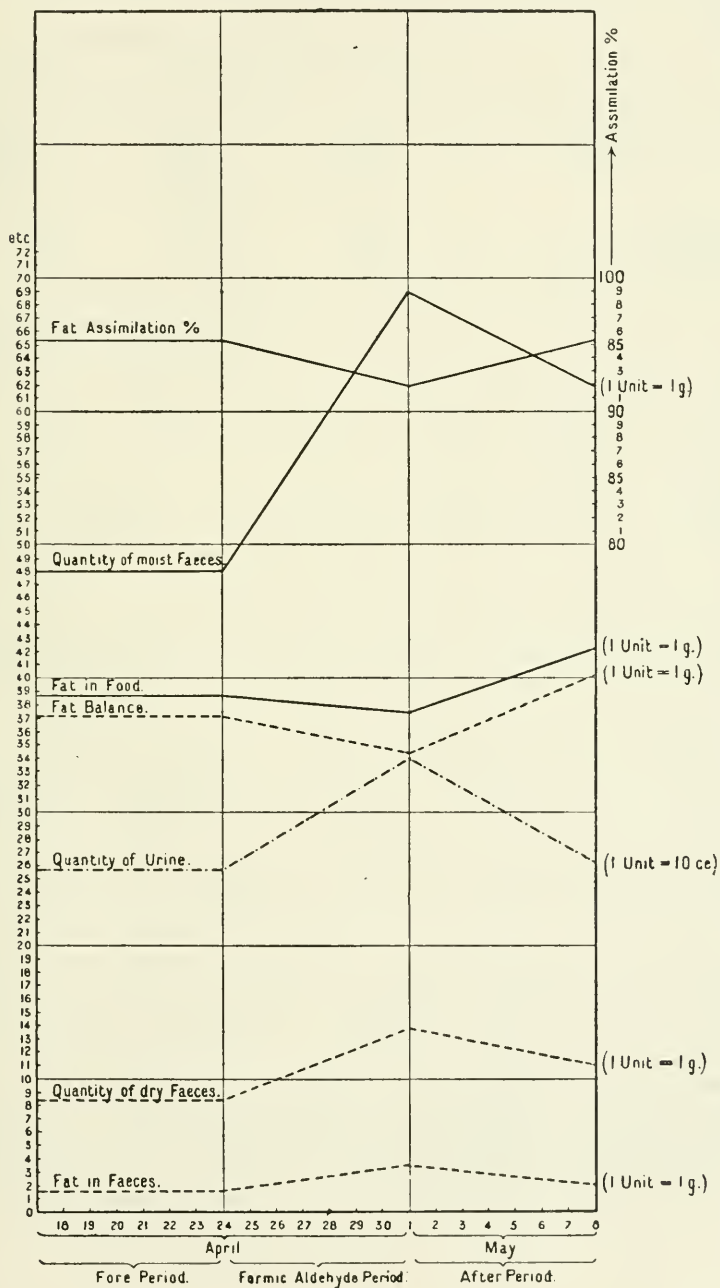
CURVE V.,

showing the influence of Formic Aldehyde upon phosphorus and nitrogen metabolism.



CURVE VI.,

showing the influence of Formic Aldehyde upon fat assimilation and upon the quantity of urine and faeces



Phosphorus Metabolism.

The results in this connection expressed in a tabular form are as follows :

—	Fore period	F.A. period	After period
Phosphorus of Food per diem ...	0·81	0·79	0·82
" " Urine " ...	0·3835 }	0·4270 }	0·3833 }
" " Faeces " ...	0·1760 }	0·3050 }	0·4087 }
Balance	+0·25	+0·06	+0·03
Assimilation %	78·28	61·39	50·16
Phosphorus % in dry Faeces ...	2·1	2·2	3·7

From these figures we come to the conclusion that the phosphorus balance, although approaching nearer to the equilibrium during the formic aldehyde and after period, never actually reached it, and hence cannot be considered to have been seriously affected. On the other hand, reasoning from the increased amount of phosphorus in the faeces, there can be no doubt that speaking generally the phosphorus of the food has not been assimilated during the formic aldehyde and after periods to the same extent as during the fore period. Further, taking into consideration the increased amount of phosphorus in the urine during the formic aldehyde period, along with the diminished assimilation and the fact that the phosphorus in the food remained constant, formic aldehyde seemed to exert a slight stimulating action upon the breaking down of the body proteids rich in phosphorus.

As this case affords the first example of tangible increase in the phosphorus of the faeces we examined it more carefully. Keeping in mind the results of the experiments *in vitro* a probable explanation appeared to be that the formalisation of the proteid constituents of the food had rendered them less susceptible to the action of the pancreatic secretion. It must be remembered however in this connection that the pancreatic juice acts upon the residue of gastric digestion, the phosphorus-containing compounds of which are chiefly nucleo-proteids, and nucleo-albumins. An indication of the extent to which pancreatic digestion was deranged would be given by an estimation of the nucleo-proteid and nucleo-albumin phosphorus in the faeces. This we proceeded to do, following generally the method described by Knöpfelmacher¹, modified by Müller². It must be noted

¹ *Wiener klin. Wochenschr.*, 1898, No. 45.

² *Zeitschr. f. Biologie*, 1900, p. 451.

at once however that this method is not an absolute analytical one, but simply comparative¹.

Another possible explanation of the increased amount of phosphorus in the faeces would be an increased excretion of lecithin with them. Our attention was drawn to the possibility of this explanation by the fact, as will be seen later, that the substances soluble in ether in the faeces were actually increased during the formic aldehyde period. We proceeded therefore to estimate the phosphorus due to lecithin, and found it contrary to our expectation to have considerably decreased during the formic aldehyde period.

Having obtained the figures for phosphorus due to lecithin and due to nucleo-proteids, we were enabled by subtraction of their sum from the total phosphorus to obtain a phosphorus value representing in all probability inorganic phosphorus.

These results in a tabular form are as follows :

—	Total P.	Nucleo-proteid P.	Lecithin P.	Inorganic P.
Fore period	0·1760	0·0085	0·0226	0·1459
Formic Aldehyde period ...	0·3050	0·0178	0·0086	0·2786
After period	0·4087	0·0149	0·0264	0·3674

It will be seen from the above table that the nucleo-proteids of the faeces as measured by their phosphorus content are increased, and from this we are justified in concluding that formic aldehyde has exerted some influence upon their digestion. Since this effect is continued into the after period the conclusion seems justified that it is due rather to a specific action upon the secretion of the pancreatic enzymes than to a diminished digestibility of the food. Whatever explanation of the increase in nucleo-proteids we may adopt, this latter is not sufficient of itself to explain the actual increase in the total phosphorus; much less does the lecithin afford an explanation of this. We are forced therefore to assume that the increased phosphorus excretion is due mainly to an increased excretion of what we have termed inorganic phosphorus. A further confirmation of this conclusion would be found in an increased excretion by the faeces of those bases with which

¹ The faeces (about 5 g.) freed from fat were ground up with 200 c.c. of dilute HCl containing some tannic acid, to fix the nucleo-proteid phosphorus. After 24 hours' standing the mixture was filtered, and washed with the tannic acid HCl solution till 100 c.c. of the washings contained no trace of phosphoric acid. Phosphorus was then estimated in the residue by Neumann's method described under general methods.

phosphoric acid is usually combined in an insoluble form, viz., Calcium and Magnesium. A quantitative analysis of the faeces in this respect showed an actual increase, proportional to that of the inorganic phosphorus, in these bases during the formic aldehyde and after periods. The results are expressed in the following table:

—	% Ash of Faeces	Absolute Amount		% CaO of Ash	% MgO of Ash
		CaO per diem	MgO per diem		
Fore period	22.11	0.6654	0.0674	3.58	0.36
Formic Aldehyde period	32.51	1.2139	0.1162	3.96	0.38
After period	20.77	0.8688	0.0829	3.84	0.36

Taking all these results into consideration, we should be inclined to conclude that the increase of phosphorus in the faeces depends upon an increase in inorganic phosphorus due to the co-operation of two causes. Firstly to a stimulating action exerted by formic aldehyde, or more probably formic acid, upon the intestinal secretion, and secondly to a less extent to an increased splitting up of lecithin and the transformation of the glycerophosphoric acid produced, into insoluble phosphates¹.

Fat Assimilation.

The results are as in former cases represented in tabular form:

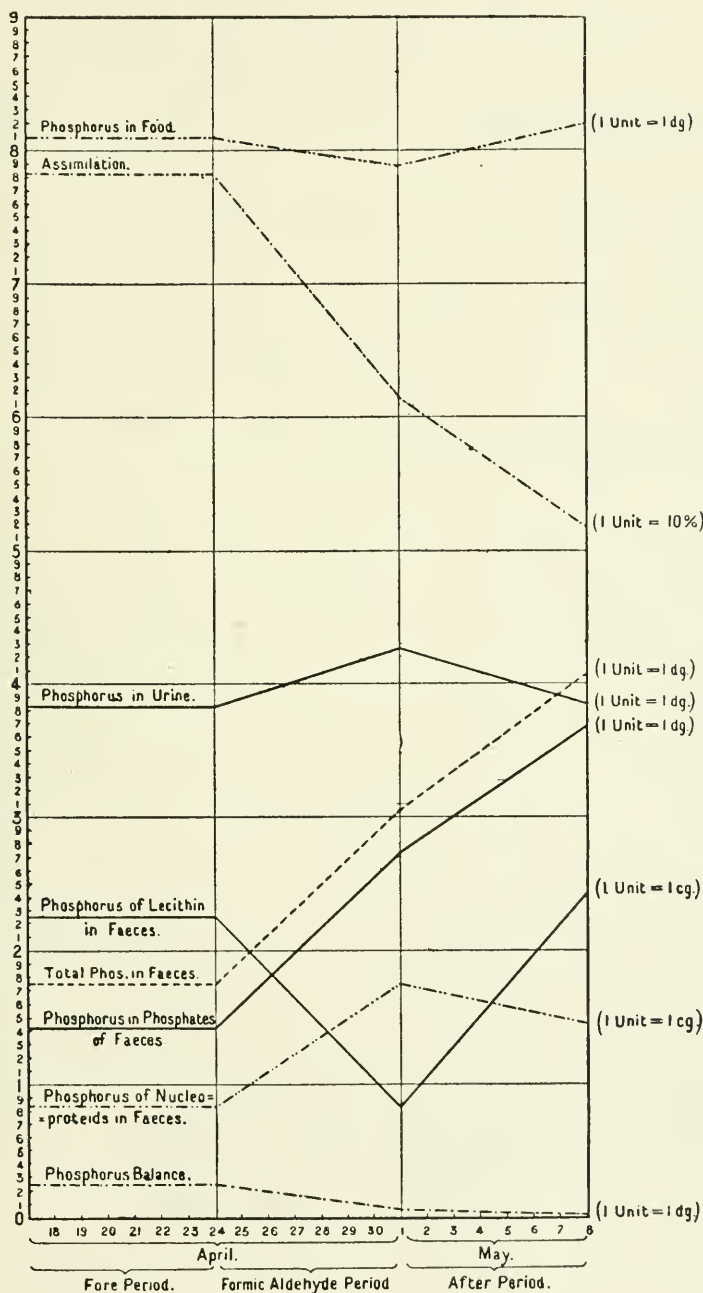
—	Fore period	F.A. period	After period
Fat in Food	38.95	37.59	42.06
„ Faeces	1.77	3.10	1.95
Balance	+ 37.18	+ 34.49	+ 40.11
Assimilation %	95.46	91.75	95.37
Fat in dry Faeces %	21.1	22.9	17.7

It will be seen from these figures that formic aldehyde interfered in this case with the assimilation of the fat of the food. The interference did not extend into the after period. From this latter fact we may conclude that in this case formic aldehyde exerted a specific action upon the fat-splitting enzyme of the pancreas.

¹ Noël Paton (*Journ. of Phys.*, 1900) found that the calcium salt of glycerophosphoric acid administered to a goat was excreted in the faeces as inorganic phosphate.

CURVE VII.,

representing graphically the phosphorus metabolism under Formic Aldehyde in a delicate child.



With regard to lecithin, we have already given the phosphorus corresponding to the lecithin, but following our previous procedure we now give the percentage of lecithin in the total fat.

—	Fore period	F.A. period	After period
Lecithin in grammes of 100 g. fat	21.15	7.25	24.77

In this case the effect on the lecithin excretion is most marked: we are inclined to ascribe this decrease and that which took place in the other cases to formic aldehyde having exerted a stimulating effect upon the lecithin-splitting ferment of the pancreas, which according to Bokai¹ splits up lecithin into glycerophosphoric acid, free fatty acids and cholin. That the diminished excretion of lecithin is not due to a retention of lecithin in the body by a direct absorption of it as such, may be concluded from the phosphorus balance.

On referring to the chief table, we see that the *quantity of urine* in this case was increased in the formic aldehyde period, an exactly opposite effect to that observed elsewhere. The specific gravity of the urine fell during this period. With regard to the excretion of *uric acid*, also an opposite effect was produced, viz., an increase. As in the after period the average uric acid figure fell below the fore period level, we infer that either a dissolving out of uric acid from the tissues took place under formic aldehyde, or a stimulated production of it. The *total sulphuric acid* excretion was somewhat increased both during the formic aldehyde and the after periods; this together with a slightly increased total nitrogen excretion suggest an effect upon general proteid katabolism in the inverse sense to that observed in Child A. The decrease in *etheral sulphuric acid* is too small to enable us to draw any conclusions other than negative ones from it. The indoxyl test remained constant throughout the whole observation. During the formic aldehyde and the after period the *body weight* increased, and this cannot be explained in this case as in the preceding ones by a retention of water in the body. This latter occurrence together with the fact that the general health of the child remained unaffected during the formic aldehyde period must tend to minimise any adverse deductions which may be made from the above results.

¹ *Zeitschr. f. physiol. Chem.*, 1877, i. p. 162.

These observations may be summarised in tabular form as follows :

TABLE IV A.

—	Nitrogen assimilation %	% N. of dry faeces	Phosphorus assimilation %	% P. of dry faeces	Fat assimilation %	% fat of dry faeces	$\frac{A}{B}$ *	$\frac{N}{SO_3}$ †
Fore period ...	91.88	6.4	78.28	2.1	95.46	21.1	12.0	6.1
Formic Aldehyde period }	89.01	5.7	61.39	2.2	91.75	22.9	14.5	6.1
After period ...	90.27	6.2	50.16	3.7	95.37	17.7	15.8	6.1

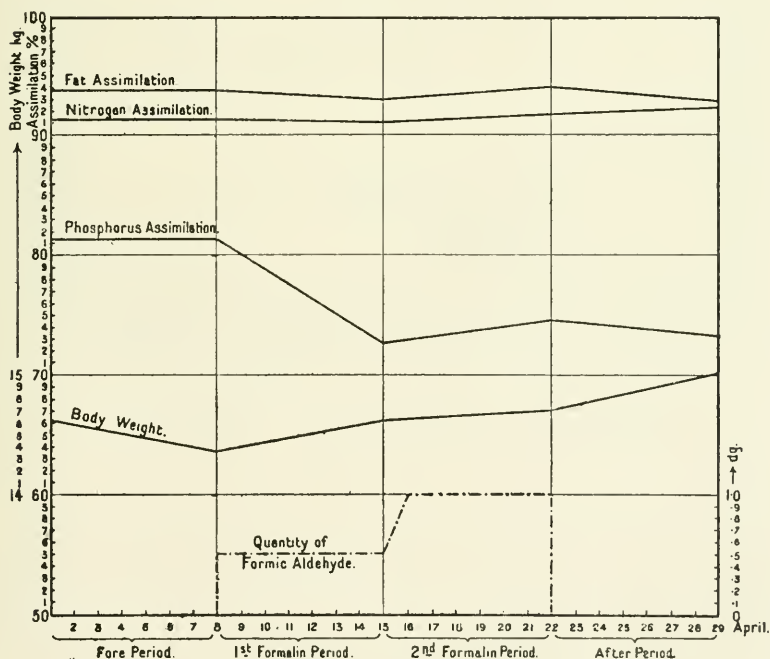
* As in Table II A.

† As in Table II A.

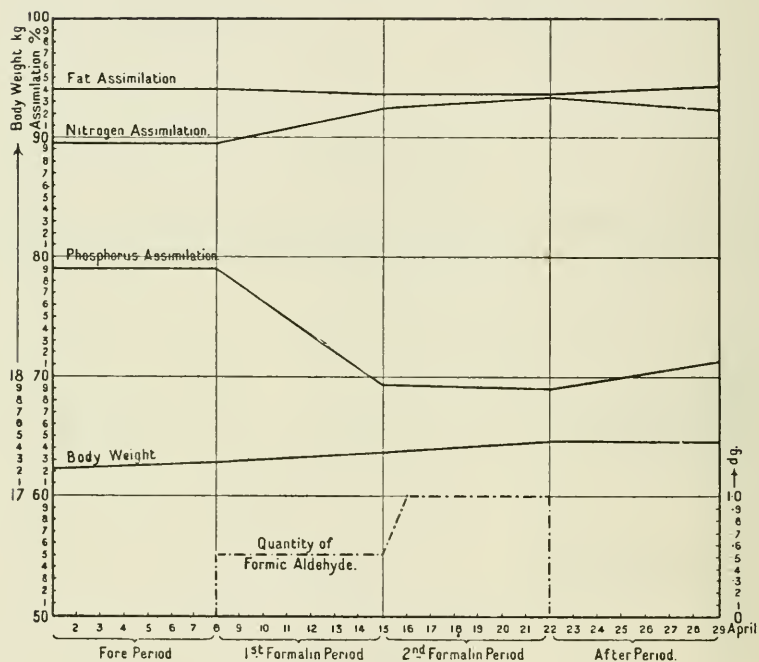
CURVE VIII.,

showing the influence of Formic Aldehyde upon the body weight and upon the nitrogen, phosphorus and fat assimilation of three children.

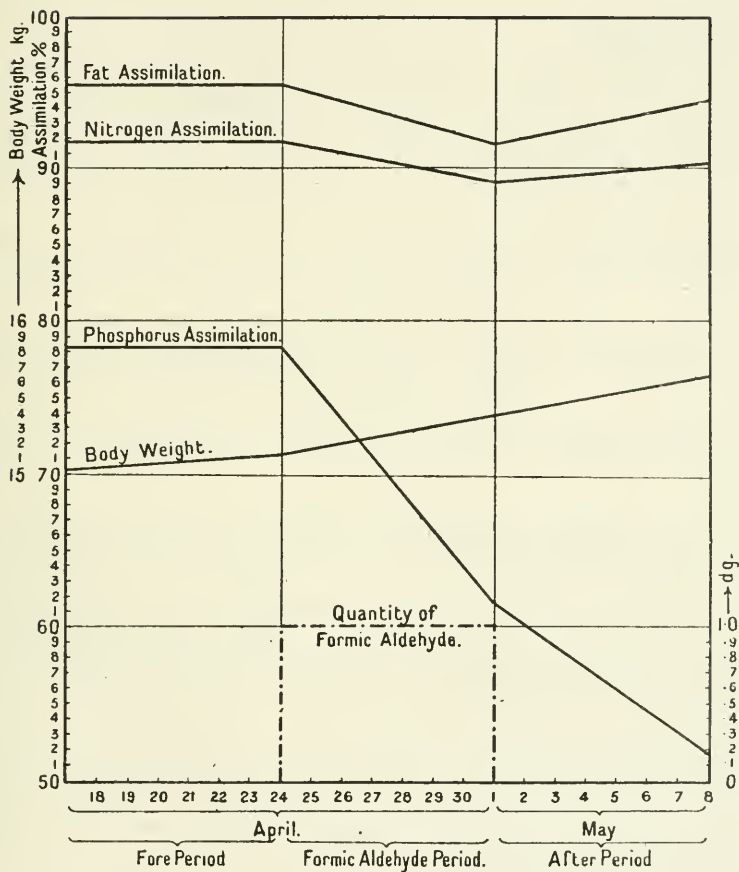
CHILD A.



CHILD B.



CHILD C.



GENERAL CONCLUSIONS.

(1) In healthy children formic aldehyde administered with the food in doses up to 1:5000 in milk or 1:9000 in total food and drink exerted no appreciable effect on the nitrogen or phosphorus metabolism or fat assimilation.

The analytical figures suggest, however, that formic aldehyde has a tendency to diminish phosphorus and fat assimilation, and hence it may be inferred that in larger doses, or if continued for a longer period, it would act in this direction. This effect is referable to an influence upon pancreatic digestion.

(2) In healthy children formic aldehyde in the above doses produces a retention of water in the body.

(3) In a delicate child formic aldehyde in the above maximum dose had a chemically measurable deleterious effect upon the nitrogen, phosphorus, and fat assimilation, again referable to an action upon the pancreatic digestion, combined with a slight intestinal irritant action. There was a slight tendency to stimulate the katabolism of proteid material.

(4) In a delicate child formic aldehyde increased the volume of urine and the weight of faeces.

(5) In all cases the excretion of lecithin in the faeces was diminished under the influence of formic aldehyde. This effect is probably referable to a stimulating action of formic aldehyde on the lecithin-splitting ferment of the pancreas.

(6) In no instance did formic aldehyde exert any appreciable intestinal antiseptic action.

(7) In no instance was there any influence on the general health or well-being of the children.

KING'S COLLEGE, LONDON.

January 1901.

ON THE FORMATION OF SPECIFIC ANTI-BODIES IN
THE BLOOD FOLLOWING UPON TREATMENT WITH
THE SERA OF DIFFERENT ANIMALS, TOGETHER
WITH THEIR USE IN LEGAL MEDICINE.

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DURING the thirteen years which have elapsed since the publication of my researches upon the bactericidal properties of the blood and other fluids of the body¹, a large amount of most valuable knowledge has been acquired regarding the blood both in health and disease. Through the work of many investigators the complicated subject of immunity is gradually being solved, and we are beginning to understand the way in which the body combats or is protected against the inroads of disease. We have learnt of the existence of specific antitoxic, agglutinative, haemolytic, bactericidal and cellulicidal properties in the blood-serum, etc., as also of a number of neutralizing bodies to these. The quite recent discovery of specific precipitins, which act upon various bacterial products, milks, peptone, egg-albumin, and upon human blood and its derivatives, has opened a wide field for investigation, which cannot fail to ultimately yield results of the greatest importance. We have to thank bacteriological investigation for the greater part of the advances which have been made, although a considerable portion properly belongs in the domain of physiology.

¹ Nuttall, G. H. F. (5 July, 1888), Experimente über die bacterienfeindlichen Einflüsse des thierischen Körpers. *Zeitschr. f. Hygiene*, Bd. iv. pp. 353—394. (One plate.)

Before considering my own experiments regarding the anti-bodies or precipitins produced in the body experimentally I shall briefly describe the work which has been done on the subject. The majority of the papers referred to have appeared during the course of our investigations, which were commenced in January, and we find that we have been able to extend and confirm, in some cases quite independently, the observations of others.

The Action of Specific Precipitins upon certain Bacterial Filtrates.

The existence of specific precipitins in the blood was first demonstrated by Kraus¹ (12 Aug. 1897), who added cholera, plague, and typhoid anti-sera to filtrates of the cultures of the corresponding germs. The bacterial filtrates were prepared by means of stone-filters. On adding an anti-serum to an homologous culture-filtrate a precipitate was formed, but this did not happen if the anti-serum was non-homologous. A filtrate of crushed cholera germs gave the same result as the fluid-culture filtrate, proving that the substances acted upon were contained in the bacterial cell. This accounts according to Kraus, for the results of Widal, Levy and Bruns, who produced immunity by means of cholera and typhoid culture-filtrates, the immunified animals yielding an anti-serum which agglutinated the corresponding germ. On the other hand, a filtrate of diphtheria culture gave no precipitate on the addition of antitoxic horse-serum. These observations were confirmed by Nicolle (March, 1898) who worked with cultures of *B. coli*, *B. typhi*, and *Vibrio massachusetts*. He obtained most precipitate from old cultures. The reaction was not impeded by the addition of antiseptics.

Tchistovitch states that Marmorek obtained a similar precipitation when he added anti-streptococcic serum to filtered cultures of the *Streptococcus*.

The Action of Specific Precipitins on various kinds of Milk.

We are indebted to Bordet (March, 1899) for the first observations upon the production of specific anti-bodies which act on milk. Having partially sterilized milk by exposing it to a temperature of 65° C., he injected it at intervals, intraperitoneally, into rabbits. After the rabbits had been treated for some time they were bled, and it was found that

¹ See Bibliography at the end of this Paper.

their blood-serum had acquired a specific precipitin which acted only upon the particular milk against which the animal had been immunified. In testing, he placed about 3 c.c. of the anti-serum in a tube; control tubes receiving a like quantity of normal rabbit-serum. Six to fifteen drops of milk were then added to the serum. The mixture with normal serum remained diffusely white, that with anti-serum soon showed granules and subsequently a definite precipitate.

The experiments of Bordet were repeated by Wassermann and Schütze (2 July, 1900 and 29 Jan. 1901), who treated rabbits with human, goat, and cow's milk, obtaining in each case a specific anti-serum. The rabbits treated with human milk yielded a serum which precipitated the casein of human milk, but not the casein of the other milks tested, and *vice versâ*. Schütze (29 Jan. 1901), who published the details of these experiments, dwells upon the hygienic importance of this method in the study of the chemistry of milk, especially nowadays, when efforts are being made to simulate mother's milk. He cites the observations of Fisch (Feb. 1900) in America, the results of the latter being of an analogous nature. Fisch found that an emulsion of udder-cells, injected into animals, gave the same results as when milk was injected, another proof for the theory that milk is not simply a filtration-product of the milk-gland, but a true solution of the cells of that gland¹.

In the experiments of Wassermann and Schütze the rabbits were treated by subcutaneous injections of 10—20 c.c. of milk, repeated every 3 to 4 days. In some cases, doses as large as 30 to 50 c.c. were injected. The milk was sterilized by means of chloroform prior to injection. The animals were bled after 3 weeks, their serum being added in the proportion of 1:1, or 1:5, to milk diluted 1:40; the mixture being left for some hours at room-temperature. Schütze calls the anti-sera produced by milk-inoculations "*Lactosera*." According to Schütze, milk which has been boiled for half-an-hour has lost the greater part of its power to form a precipitate on the addition of its specific lactosera.

These experiments very clearly show that there are essential differences in the composition of various kinds of milk, and that heating milk alters the composition of the albuminous molecule.

¹ The original paper by Fisch has unfortunately remained inaccessible.

The Action of Specific Precipitins upon Peptone Solutions.

Tchistovitch (May, 1899) treated rabbits with injections of 10% peptone solution, the dose introduced being 5 c.c. He was unable to observe the formation of precipitins in the rabbit's serum. Myers (14 July, 1900), on the other hand, has obtained positive results with solutions of Witte's peptone. Exposed to a temperature of 56° C. for half-an-hour, the anti-serum exerted less action. The weakening due to heat was markedly counteracted through the addition of normal rabbit-serum to the heated serum, although the normal serum had no effect when used alone.

Myers considered that his results strongly support the view that the production of immunity is due to processes of assimilation, peptone disappearing from the blood as does tetanus-toxin. Just as Wassermann showed that certain organ-emulsions neutralize tetanus-toxin, so Neumeister¹ showed that peptone could be neutralized by pieces of intestine. The fact that such heated "pepto-serum," if I may be permitted the expression, could be rendered again active by the addition of normal serum, led Myers to conclude that such a serum is capable of forming precipitoids analogous to toxoids.

*The Action of Specific Precipitins upon Egg-Albumin,
Blood and Blood Derivatives.*

The credit of having first observed the formation of specific precipitins in the blood of animals treated with various sera belongs to Tchistovitch, as stated by Bordet (March, 1899). Tchistovitch (May, 1899) inoculated rabbits with eel-serum, which is toxic, and thereby obtained a specific antitoxic serum from these animals, as also from similarly treated goats, dogs, and guinea-pigs. In addition to the antitoxic property acquired by the serum it was found to acquire the property of producing a precipitate when it was added to eel-serum. When but a small amount of toxin was present the precipitin acted but feebly. Normal sera gave no such reaction. Adopting the terminology of Myers, I shall hereafter refer to the precipitate formed in a serum on the addition of its specific anti-serum as a *precipitum*.

Tchistovitch found that the precipitum was soluble in dilute acids

¹ *Lehrb. d. physiol. Chem.*, Jena, 1893.

and alkalis, but that it was insoluble in water, as also in solutions of alkaline carbonates and neutral salts. The precipitum is only formed in alkaline solutions. In neutral solutions there may be some opalescence, whilst acid solutions remain clear. The precipitum is non-toxic to rabbits by intravenous injection, although the clear fluid may be toxic if it contains unneutralized toxin. It is an interesting fact that the precipitin disappears in animals which have been treated for some time, having been rendered immune. Eel-serum heated to 58° C. gave less precipitum on the addition of its anti-serum. The reaction proved negative with eel-serum which had been heated to about 80°.

Tchistovitch next injected rabbits with horse-serum, the animals receiving 5 to 6 injections of 3 c.c. at a time. The treated rabbits yielded an anti-serum which produced a precipitum with horse-serum, but not with that of the donkey, nor of the normal rabbit. Tchistovitch draws attention to the fact that agglutinins are different bodies to the precipitins, for no precipitins are formed in the serum of animals treated with *B. tetani*, whereas these organisms are agglutinated by antitetanic serum¹.

Bordet (March, 1899) treated rabbits with intraperitoneal injections of defibrinated fowl's blood, and observed that their serum acquired great agglomerating and haemolysing power, and in addition produced a precipitum on being added to normal chicken-blood. As in Tchistovitch's experiments with eel-serum, the precipitum in this case was soluble in dilute alkaline solutions. This was subsequently (15 Nov. 1900) confirmed by Uhlenhuth in so far as he found the serum of rabbits treated by intraperitoneal injections of chicken-blood to contain a precipitin for dilute solutions of chicken-blood. Uhlenhuth states that his anti-serum exerted no effect on the blood of the horse, ox, sheep, and pigeon. Bordet however (p. 233) states that the anti-serum for chicken-blood *does* produce a precipitum with pigeon-blood².

Bordet states that when guinea-pigs are treated with rabbit-serum no anti-body is formed. Nolf (May, 1900) has made a similar observation on pigeons treated with chicken-blood. It seems therefore that precipitins are not always formed in the bodies of animals treated with different sera.

Nolf collected about 10 c.c. of chicken-blood by allowing it to flow into 3 to 4 times its volume of 1% salt solution. After separating the

¹ See also Bordet (1899, p. 233) and the results of Myers (1900) quoted below.

² See analogous results with experiments on chicken and pigeon egg-albumin to be mentioned presently.

corpuscles from the plasma he injected these separately into two sets of rabbits. The animals received 4 to 6 injections at intervals of 4 to 5 days. Only the serum-treated rabbits yielded a precipitin for chicken-blood, the serum of the others had no more effect on chicken-blood than has normal rabbit-serum. Two other series of rabbits treated respectively with a dog's blood corpuscles and serum gave identical results, specific precipitins being formed in serum-treated rabbits, which had only a precipitating action on dog's blood.

Nolf obtained an anti-serum for rabbit-blood by treating chickens with their serum. He confirmed the observation of Tchistovitch that horse-serum produces an anti-serum when injected into rabbits.

Nolf saturated anti-serum with magnesium sulphate, and purified the globulin precipitate by redissolving it, and afterwards reciprocating it with the same salt. He obtained the albumin by adding 1% acetic acid to the filtrate from the globulin precipitate. He removed the magnesium salts by dialysis during eight days with chloroform-water. He then added 1% NaCl to the neutral fluid, and sterilized it by exposure for half-an-hour to a temperature of 56° C. on eight successive days.

Rabbits treated with globulin-solution yielded a precipitin, whereas those treated with albumin-solution did not. Consequently the precipitum is due to the action of bodies formed in animals as the result of the reaction produced by globulin-injections. The chemical treatment to which Nolf subjected the serum evidently did not materially alter the composition of the globulin, for the artificial globulin-solution produced an anti-serum of the same character as that resulting from treating the animals with normal serum. By adding anti-serum to albumin and globulin-solutions, as also to mixtures of these, Nolf was able to prove that the precipitum is a globulin.

Myers (14 July, 1900) injected proteids (crystallized egg-albumin from the white of the fowl's egg, sheep and bullock serum-globulin, and Witte's peptone) intraperitoneally into rabbits. The crystallized egg-albumin was prepared by the method of Hopkins and Pinkus¹. Specific precipitins for solutions of egg-albumin were formed in the serum of the rabbits after they had been treated for some months. The precipitum was formed more rapidly at 37° C. than at room-temperature. A slight precipitum was produced in solutions of the egg-albumin of the duck.

¹ *Journ. of Physiol.*, vol. xxiii.

Specific precipitins also appeared in the serum of rabbits treated with sheep and ox-globulin solutions. The serum of the rabbits treated with sheep-globulin was found to contain two agglutinating bodies, the one acting on sheep-corpuscles, the other on those of the fowl. The precipitin obtained by treatment with sheep-globulin had a slight effect on ox-globulin, and the converse. On the other hand, the serum from rabbits which had been treated with ox-globulin did not agglutinate the corpuscles of either sheep or ox-blood.

The action of the precipitins in the serum of rabbits treated with the egg-albumin and globulins was not appreciably affected by exposure for half-an-hour to a temperature of 56° C. We have already referred above to Myers' experiments with peptone.

Uhlenhuth (15 Nov. 1900) diluted the white of fowls' eggs in normal salt-solution and injected the fluid in quantities up to 100 c.c. intraperitoneally into rabbits. The animals did not suffer from the treatment. After a rabbit had received 5 to 6 eggs in this way, its serum was found to contain a precipitin which acted on 5 to 10% solutions of chicken egg-albumin. The animals which had received the greatest number of eggs gave the most powerful anti-serum; in one case a positive reaction was obtained with a 1:100,000 dilution of egg-albumin. The ordinary chemical tests only gave a reaction with dilutions up to 1:1000. Normal rabbit-serum never produced this reaction, and the anti-serum added to various commercial albuminous preparations, outside of those derived from the fowl's egg, constantly gave negative results. The anti-serum, heated for one hour to a temperature of 60° C., gave almost as powerful a reaction as the unheated serum. The reaction is not entirely specific, as the pigeon's egg gave the same reactions, though to a lesser degree. Rabbits treated with solutions of pigeon egg-albumin gave a serum which also acted on chicken egg-albumin, and for this reason we may conclude that the albuminous constituents in both species of eggs are closely allied.

Uhlenhuth moreover made the interesting observation that the precipitin appeared in the blood of a rabbit which was *fed* on egg-albumin, the solution being introduced daily by means of a sound. The precipitin only appeared after this mode of feeding had been continued for 24 days.

Leclainche and Vallée (25 Jan. 1901) treated rabbits by intravenous injections of 20 c.c. of albuminous urine, containing 1 to 2 g. of albumin per liter. At times there were symptoms of intoxication, and the first

injections were not infrequently followed by emaciation. After having been treated for three months, and having received a total of 150 to 200 c.c. of urine, the animals were allowed a rest of 15 days before being bled. The serum of these animals, added to an equal volume of the urine used for treatment, produced almost immediately an albuminous precipitum. The washed precipitum gave all the albumin reactions. No precipitum was formed on the addition of the normal sera of the horse, donkey, sheep, and ox. The amount of precipitum does not depend upon the amount of albumin present in the urine, and although they obtained a precipitum with albuminous urine which had been diluted 1 : 5 or 1 : 10, the best results were obtained by mixture of equal volumes of the urine and test-serum. When heated for 2 hours at 58° C. the test-serum was still effective, whilst the albuminous urine, thus treated, showed a much less marked reaction.

The anti-serum was very active for serum-albumin, but almost indifferent towards urine containing much globulin. A positive reaction was obtained with the urine of three cases of interstitial nephritis, but there was no reaction when the test-serum was added to the urine of a case of parenchymatous nephritis which contained much globulin. Albuminous urine from the horse and cow gave no reaction. A positive reaction was obtained with human pleuritic exudation, but not with human blood-serum.

Uhlenhuth (7 Feb. 1901) injected rabbits intraperitoneally with 10 c.c. of defibrinated ox-blood. After 5 to 6 injections, made at intervals of 6 to 8 days, their serum gave the specific reaction. Bloods of different animals were diluted 1 : 100 with tap-water, the remains of the stroma were removed by sedimentation or filtration, and about 2 c.c. of the clear solution were placed in small tubes about 6 mm. wide. An equal volume of double normal salt-solution (1.6 %) was then added to the blood-solution, this being essential as normal rabbit-serum produces clouding of the watery blood-solution, and this may mask the result. Uhlenhuth used clear solutions of human blood and that of the ox, horse, donkey, pig, sheep, dog, cat, deer, fallow-deer, hare, guinea-pig, rat, mouse, rabbit, chicken, goose, turkey, and pigeon. On adding 6 to 8 drops of ox-blood anti-serum to the various blood-solutions he only obtained a positive reaction with ox-blood.

Similar results were obtained by treating rabbits with human blood, the test-serum in this case only giving a reaction with human blood. He moreover made the important observation that human, horse, and ox-blood which had been dried for 4 weeks on a board, could be readily

distinguished by the test-serum, added to these bloods in normal salt-solution.

As Wassermann and Schütze (18 Feb. 1901) point out, the reactions produced by specific haemolysins and agglutinins are of but little value forensically, for to obtain these reactions a large number of *intact* blood-corpuscles must remain in suspension.

They confirmed the observations of previous investigators in that they obtained anti-sera from animals treated with human and other bloods. They treated the animals by injecting 10 c.c. of the particular blood-serum into rabbits, the injections being made subcutaneously about every two days. After the rabbits had received 5 to 6 injections a period of six days was allowed to elapse, after which they were bled to death, and the blood was placed in the ice-chest. The precipitum was formed more rapidly at 37° than at room-temperature.

Wassermann and Schütze tested their anti-sera on 23 kinds of blood: that of the horse, donkey, goat, cow, ox, sheep, pig, dog, cat, baboon, guinea-pig, rabbit, house-mouse, house-rat, goose, duck, chicken, sparrow, eel, pike, and tench. On adding the test-serum obtained from rabbits treated with human blood, none of the bloods reacted except human and baboon blood. The blood of the baboon, however, reacted much more slowly and incompletely to the test, than did the human blood.

Tests were moreover made with blood which had been allowed to dry on knives, linen, etc. The blood which had been dried for 3 months, was dissolved in normal salt-solution, and filtered, so as to obtain a clear solution. About 5 to 6 c.c. of salt-solution were added to a dried drop about the size of a sixpence, and about 0.5 c.c. of the test-serum were added to the blood-solution. The reaction was well marked after 20 minutes at 37° C. They state that the reaction was more powerful with fresh anti-serum than with serum which had been preserved for some time; nevertheless, a positive reaction was obtained with a test-serum which had been kept for two weeks on ice.

Stern (28 Feb. 1901) reports similar experiments. He injected rabbits every two or more days, according to the health of the animal, the dose of serum being 5 to 10 c.c. After 2 to 3 weeks the serum of the rabbits contained a precipitin, which only acted on human blood, whether fluid, or dried and made into solution, and on albuminous urine. The result was negative when the blood-serum of the horse, ox, sheep, and pig were tested. He also found the reaction feeble though

positive with the blood of three species of monkeys, a species of *Cercopithecus* ("Meerkatze"), *Macacus cynomolgus* L. ("Java-Affe"), and the "Kronen-Affe." According to Stern the reaction is therefore not strictly specific.

By long-continued treatment the amount of precipitin can be greatly increased. Stern states that he has a rabbit the serum of which gives a positive reaction when added to human blood diluted 1:50000.

Mertens (14 March, 1901) treated rabbits by intraperitoneal injections of egg-albumin of the fowl, as also subcutaneous and intravenous injections of human blood-serum, his results confirming those of the previous observers. He also treated rabbits with albuminous urine, but found that he obtained a greater amount of precipitum when he used the serum-treated animals' blood, instead of that from rabbits which had been injected with albuminous urine. This was doubtless due to the greater amount of the anti-body produced by serum-injections. He concludes that the albumin in urine must be derived from the blood, for otherwise it would not be acted on by the precipitins in the blood of serum-treated animals. He made the interesting discovery that the blood of a young rabbit, born of a serum-treated mother, gave the typical reaction.

Dieudonné (2 April, 1901) treated rabbits by means of human albuminous urine, pleural exudation, and blood-serum. He injected quantities of 10 c.c. every 3 to 4 days. He made his tests according to Uhlenhuth's method, and found that he obtained no reaction with four other bloods, i.e. of the rabbit, guinea-pig, pigeon, and goose. Though the animals had been treated in various ways, they all gave practically the same test-serum. Normal rabbit-serum had no effect on human blood-solutions.

We come finally to the observations of Zuelzer (4 April, 1901). He made the same experiments as Mertens. He injected 5 to 10 c.c. of albuminous urine into rabbits every day to every third day, the rabbits yielding a precipitin at the end of 2 to 3 weeks. The urine he injected contained 1 to 9 ‰ albumin. Though this proves that one albuminous body in the tested urine was derived from the blood, he does not consider that it justifies Mertens' generalization that all albuminous bodies in nephritic urine are derived from the blood.

Methods.

In the following experiments only rabbits were used for obtaining the specific anti-sera. The blood, pleuritic exudation, etc., was injected intraperitoneally in quantities of 5 and 10 c.c., usually beginning with the smaller dose, the amount of 10 c.c. not being surpassed. Only a few injections were made subcutaneously, intraperitoneal injection being preferred. The animals were so little affected by the operation that the males frequently sought to copulate with the females immediately after being released from the hands of the assistant, who held them belly upwards on a high stool. The abdomen was shaved over the seat of operation, and the skin disinfected with lysol. The skin was punctured by means of a small scalpel in the lower left-hand region of the abdomen. The somewhat blunted hypodermic needle was then introduced through the puncture and gently bored through the abdominal wall, through which it passed with a jerk. After the injection had been made, and the needle withdrawn, the parts were dried with sterilized cotton, there being no bleeding, and tincture of benzoin was applied over the small slit (usually 1.5 to 2 mm. long) in the skin. The syringe used was entirely made of metal, the piston being lubricated with sterilized vaseline. It was disinfected before use by means of lysol solution, which was also used for rinsing it out, when several injections were made in succession with the various sera, and into different animals. The animals were tattooed upon the inner side of the ear with a letter and number, the letters *D*, *H*, etc. indicating the kind of blood injected. This excluded any possible confusion. The animals were regularly weighed every day, or every second day, and the injections were not repeated until they had regained any lost weight. The loss in weight was generally small and usually less after the first two injections.

We only lost three animals; in one, a young animal, death was due to PsorospERMiasis; in the second the cause of death could not be determined at the autopsy. The third animal (ox-serum-treated rabbit No. II, see Protocol) died of an inter-current disease. There was no peritonitis or bacterial infection in any of these cases. We never observed any symptoms of intoxication consequent upon the serum-injections, the sera used being those of the dog, ox, sheep, horse, cat, and man, besides human pleuritic exudation.

Effective anti-sera were obtained after the fifth or sixth injection,

sometimes earlier, as will be seen from the Protocols. The animals were periodically bled by puncturing the lateral ear-vein, the skin having been shaved, disinfected with lysol, and dried with sterilized cotton. The blood, as it flowed from the vein, was collected in fine-pointed sterilized bulbed pipettes from which it was expelled into test-tubes which were laid almost horizontally into racks.

We obtained a considerable quantity of serum in this way from small amounts of blood, the tubes being placed vertically in test-tube racks after the coagulum had formed. Ten to forty c.c. of blood were readily obtained from the ear-vein by this method. In other cases the rabbits were bled to death by cutting the carotids and catching the blood in large flat dishes and Petri-dishes, these being subsequently tilted and the serum placed in corked bottles to which chloroform was added. This method served the purpose practically as well as when we pipetted off the sterile serum from the test-tubes and sealed it in glass tubes. We have found that neither drying for some weeks, nor the addition of chloroform to anti-sera and normal sera, prevents the reaction taking place.

Normal serum was sometimes sterilized by filtration, and preserved in sealed tubes. At other times it was dried and solutions of the dried substance were injected. Sera which had been collected with ordinary precautions as to cleanliness, and to which lysol or chloroform had been added, were also used. In the last case the serum that was to be injected, was poured into sterile Petri-dishes which were placed uncovered in the thermostat until the chloroform had evaporated.

We have found it very convenient to preserve normal serum and defibrinated blood on strips of filter-paper, which we have immersed in blood and hung up to dry, pinning them to the edge of a table or bench. It is best to keep one end of the strip clean, so as to make pencil-notes upon it. It is possible to roughly estimate the amount of blood or serum absorbed by these strips, and to cut out squares of suitable size for purposes of testing. Whereas the blood and serum which is dried on horizontal glass plates in the incubator-room only dissolves slowly and frequently gives clouded solutions, the filter-paper rapidly gives off the soluble ingredients of the blood in perfectly clear solution, the fibres of the permeable paper retaining the fine particulate matter and allowing the solvent to act rapidly. We have also preserved some of our anti-sera in this way.

In the following protocols the weights of the animals are given thus, "2500—2490 g.," this signifying that the weight at the beginning of the

experiment was 2500 g., and at the conclusion 2490 g. The test for specific precipitins was made by adding about 3 drops of the treated animal's serum to a clear filtered 1:100 dilution of the blood, etc. with which it had been treated. Fluid serum was diluted with normal salt-solution. Dried serum was dissolved in ten parts of normal salt-solution and this diluted 1:100. Dry blood was dissolved by means of water, as recommended by Uhlenhuth, to which an equal quantity of double NaCl solution (1.6 %) was subsequently added. The results of the tests are given in the following protocols of our experiments. Where much precipitin was formed we refer to the reaction as "marked," etc. The test-tubes used contained about 0.5 c.c. of blood-dilution. When test-serum flowed to the bottom of the tube the reaction was most striking at the line of contact between the fluids.

I. *Rabbits treated with Dog-Serum.*

I. Weight 2460—2410 g. Treatment lasted 51 days. Received 6 injections, the first of 5 c.c. subcutaneously, the rest of 10 c.c. intraperitoneally, the last injection being made one week before the animal was bled to death from the carotids.

Bled 20 c.c. from ear after injection 3. *Marked reaction.*
 „ 20 c.c. „ „ „ 5. „ „

II. Weight 1720—1850 g. Under treatment 50 days. Received 7 injections, the first and fifth of 8 c.c., the rest of 10 c.c. intraperitoneally.

Bled 10 c.c. from ear after injection 3. *Reaction.*
 „ 15 c.c. „ „ „ 4. *Marked reaction.*

The serum obtained from the first bleeding contained less precipitin than that from the second.

III. Weight 2300—2170 g. Under treatment 51 days. Received 6 injections, the first and third of 6 c.c., the rest of 10 c.c., intraperitoneally. Bled to death from carotids seven days after last injection.

Bled 15 c.c. from ear after injection 3. *Reaction.*
 „ a few c.c. „ „ „ 5. *Marked reaction.*

The reaction obtained with the serum obtained from the second bleeding was much greater than at the first bleeding.

II. *Rabbits treated with Sheep-Serum and Defibrinated Blood.*

I. Weight 2270—2030 g. Under treatment 66 days. Received 7 intraperitoneal injections, the first of 5 c.c. defibrinated blood, the second of 5 c.c. fluid serum, the third of 7 c.c. normal solution of dried serum, the rest of 10 c.c. filtered serum.

Bled 25 c.c. from ear after injection 4. *Very slight reaction.*
 „ 4 c.c. „ „ „ 7. *Marked reaction.*

II. Weight 2220—2970 g. Under treatment 66 days. Received 6 injections, the first intraperitoneally of 5 c.c. defibrinated blood, the second subcutaneously of 7 c.c. filtered serum, the third of 10 c.c. normal solution of serum, the rest of 10 c.c. filtered serum intraperitoneally. Bled to death from carotids.

Bled 15 c.c. from ear after injection 4. *Marked reaction.*

„ 15 c.c. „ „ „ 5. „ „

There was no appreciable difference between the reactions produced by the serum from these two bleedings, when added to a 1 : 100 dilution of sheep's blood.

III. *Rabbits treated with Ox-Serum.*

I. Weight 1970—1700 g. Under treatment 66 days. Received 9 injections, the first of 5 c.c. intraperitoneally, the second of 7·5 c.c. subcutaneously, the rest of 10 c.c. intraperitoneally. Injections 5 and 6 were made with normal solution of dried serum, the last three with serum preserved with chloroform.

Bled a few c.c. from ear after injection 5. *Marked reaction.*

„ 25 c.c. „ „ „ 8. „ „

The serum used for testing in the last case had been preserved 8 days in a sealed tube at room-temperature. It was added to a 1 : 100 dilution which had been left standing for 13 days with chloroform. There was no marked difference in the reaction produced by the first and second serum.

II. Weight 2220—1810 g. Under treatment 55 days, its weight having fallen from 1980 g. during the last 6 days. The animal was killed by being bled from the carotids. At autopsy the left kidney was found to be hydronephrotic, the spleen pale, the retroperitoneal glands much enlarged and caseous. The bacteriological examination was negative.

Bled 35 c.c. from ear after injection 4. *Marked reaction.*

„ 10 c.c. „ „ „ 7. „ „

The reaction obtained with the second serum was somewhat more marked.

III. Weight 2500—2490 g. Under treatment 66 days. Received 7 intraperitoneal injections, the first of 5 c.c., the second of 7·5 c.c. (in part subcutaneously), the fourth of 7 c.c., the rest of 10 c.c. Injection 5 was made with normal solution of serum, the last two with serum preserved with chloroform.

Bled 10 c.c. from ear after injection 5. *Marked reaction.*

IV. *Rabbits treated with Horse-Serum.*

I. Weight 1370—1700 g. Under treatment 30 days. Received 6 intraperitoneal injections, the first of 5 c.c., the rest of 10 c.c. This animal was treated entirely with an old weakly antitoxic serum for diphtheria, preserved with 0·4% trikresol in a corked bottle for 2 years and 7 months.

Bled 6 c.c. from ear after injection 6. *Reaction.*

II. Weight 2180—2170 g. Under treatment for 30 days. Received 5 intraperitoneal injections, the first of 5 c.c., the rest of 10 c.c. The first two injections were made with the old trikresol serum mentioned under Rabbit I, the rest with filtered horse-serum preserved with chloroform.

Bled 20 c.c. from ear after injection 5. *Marked reaction.*

III. Weight 1970—2150 g. Under treatment for 30 days. Received the same treatment as the preceding rabbit.

Bled 15 c.c. from ear after injection 5. *Reaction.*

V. *Rabbits treated with Cat-Serum.*

Both of these animals were treated with normal cat-serum, collected aseptically and preserved in sealed tubes until used.

I. Weight 1620—1950 g. Under treatment 58 days. Received 9 intraperitoneal injections, the first, fourth and fifth of 4, 8 and 9 c.c. respectively, the rest of 10 c.c.

Bled about 50 c.c. from ear after injection 5. *No reaction.*

„ 5 c.c. „ „ „ 9. „

II. Weight 1720—2370 g. Under treatment 58 days. Received 8 intraperitoneal injections, the first of 4 c.c., the second of 6 c.c., the rest of 10 c.c.

Bled 10 c.c. from ear after injection 4. *No reaction.*

„ 5 c.c. „ „ „ 8. „

VI. *Rabbits treated with Human Blood and Pleuritic Exudation.*

I. Weight 3070—2930 g. Under treatment 46 days. Received 6 intraperitoneal injections, each of 10 c.c. With the exception of injection 5, which was made with fresh serum, the animal only received pleuritic exudation which had been preserved for 5 to 6 months by the addition of chloroform, having been kept in a corked bottle at room-temperature.

Bled 30 c.c. from ear after injection 4. *Reaction slight.*

„ 40 c.c. „ „ „ 5. *Marked reaction.*

II. Weight 3120—3040 g. Under treatment 46 days. Received 6 intraperitoneal injections, each of 10 c.c., the third and fourth with filtered serum, the rest with the old pleuritic exudate, mentioned under Rabbit I.

Bled 20 c.c. from ear after injection 4. *Reaction.*

„ 40 c.c. „ „ „ 5. *Marked reaction.*

III. Weight 1690—1620 g. Under treatment 39 days. Received 5 intraperitoneal injections, the second of 7 c.c., the rest of 10 c.c. of filtered serum, fresh pleuritic exudation, and exudation to which chloroform had been added.

Bled 4 c.c. from ear after injection 5. *Reaction.*

IV. Weight 1524—1680 g. Under treatment 39 days. Received 6 intraperitoneal injections, the first of 6 c.c., the rest of 10 c.c. as in Rabbit III.

Bled 8 c.c. from ear after injection 3. *No reaction.*

The protocols of our experiments show that we obtained precipitins in the blood of rabbits treated with dog, sheep, ox, horse, and human blood. Our results have been negative in rabbits treated with the blood of the cat. The analogous observations of Bordet and Nolf have already been referred to on p. 371.

The rest of the animals yielded an anti-serum which produced a

marked reaction (much precipitum) after the third injection, in some the reaction only took place after the fifth injection. This does not seem to depend upon differences in the weights of the animals in relation to the dose of serum, but upon individual differences in the reacting power of the rabbits.

We have tested 36 kinds of blood up to the present¹, the bloods used being those of man, four species of monkey: *Cercopithecus campbelli* Waterh., *Cercopithecus patas*, W. coast of Africa, *Cercopithecus lalandii* Is. Geoffr. [S. Africa], *Macacus rhesus* [India], the Rufous Rat-Kangaroo (*Hypsiprymnus rufescens* [Gray], N. S. Wales), the Capybara (*Hydrochoerus capybara*, S. America), the polecat (*Mustela putorius*), Suricate (*Suricata tetradactyla* [S. Africa]), squirrel (*Sciurus vulgaris*), guinea-pig, tame and wild rabbit (*Lepus cuniculus*), white rat, black rat (*Mus rattus*), horse, ox, sheep, white-tailed gnu (*Connochaetes gnu*, S. Africa), gazelle (*Gazella arabica*), deer (*Cervus axis* Erxl.; India), dog, cat, pig, bat (*Plecotus auritus*), pigeon, chicken, pheasant, swan (*Cygnus olor*), duck, chaffinch (*Fringilla coelebs*), cross-bill (*Nucifraga caryocatactes*), rook (*Corvus fragilegus*), swallow (*Hirundo urbica*), corn-crake (*Crex pratensis*), frog (*Rana temporaria*), newt (*Molge cristata*), snake (*Tropidonotus natrix*)².

The serum of rabbits treated with *dog-serum*, added to all these bloods, gave a negative reaction throughout, excepting in the case of the dog. The tested dog-blood was dried and dissolved in salt-solution, or used in the form of diluted fluid serum. Whereas a marked and almost immediate precipitation occurred on the addition of the specific anti-serum to dog's blood, all the other blood-solutions remained perfectly clear.

The serum of rabbits treated with *sheep-serum* produced a marked precipitum with sheep-serum or blood-solution, as also a distinct but less marked reaction with the blood of the gazelle and axis deer. All the other sera and bloods remained perfectly clear, excepting those of the ox, squirrel and swan, in which there was very slight clouding.

The serum of rabbits treated with *ox-serum* only produced a marked precipitation in ox-serum dilutions, or dried ox-blood solutions. A distinct reaction was obtained with the blood of the gazelle and axis deer. All the other bloods gave a negative reaction, a slight clouding only being produced in blood-solutions of the sheep, gnu, squirrel and swan.

¹ The investigations on different bloods are being pursued and will be reported later.

² We are indebted to Frank E. Beddard, Esq., F.R.S., Prosector of the Zoological Society's Gardens, London, for nine of these bloods.

The serum of rabbits treated with *horse-serum* only produced precipitation in dilutions of horse's blood or serum, not even a clouding in any of the other bloods noted.

The serum of the rabbits treated with *human* blood, serum, and pleuritic exudation only produced a marked precipitation in human blood-solutions, etc. The blood of the four monkeys gave a slight but distinct reaction. A very faint clouding appeared in the solutions of the bloods of the horse, ox and sheep, whereas all the other bloods remained perfectly clear. The test gave positive results when made with diluted human serum, pleuritic exudation, both fresh and putrid, blood and serum which had been dried on filter-paper and on glass plates, with blood which had undergone putrefaction for two months, with the blood of several persons who had cut themselves (blood collected on filter-paper), with the serum from a blister on the foot following upon a long walk, and with the serum from a blister following a burn on the hand. Both nasal and lachrymal secretion gave a slight but decided reaction. A faint clouding was produced in normal urine. That the precipitum formed in putrid blood dilution was specific was proved by adding the anti-sera of rabbits treated with ox, sheep and dog-serum to the blood dilution, no reaction resulting.

The tests made with dried blood, whether dried on glass or filter-paper, gave us perfect reactions, as did also 1:100 dilutions kept for two weeks in test-tubes in the laboratory. Although chloroform had been dropped into the bottom of these tubes, moulds occasionally developed upon the surface of the serum, but this seemed in no way to interfere with the specific reaction. Strips of filter-paper upon which both sheep and ox-blood had been allowed to dry were placed under different conditions. Some were kept for 2 months at 37° C., in the dark; others at room-temperature in the dark, and in diffused light for the same period; others again were exposed for eight days to the action of sunlight in a window. All of these samples gave apparently just as good reactions as fresh bloods, though of course our method cannot as yet be strictly considered to be quantitative. Both the body in the serum which is acted upon by the anti-serum as also the specific body in anti-serum seem to be about equally resistant. Anti-serum dried for 42 days on filter-paper and then dissolved in salt-solution was found to give a perfectly characteristic reaction when added to its homologous (ox) serum, the latter diluted (1:100) as usual; it did not however produce a reaction in dilutions of other bloods. Dried normal sera exposed for half-an-hour to a temperature of 100° C. still gave a clear reaction, as did also 1:100 dilutions exposed for half-an-hour to 55°.

As I first showed the bactericidal properties of blood are destroyed at the latter temperature (see footnote, p. 367). Dilutions of blood exposed to a temperature of 100° gave no reaction.

The first rabbit in the series treated by horse-serum injections received old antitoxic serum which had been kept at room-temperature in the laboratory for *two years and seven months*. We are indebted to Dr Louis Cobbett for this serum. The serum, to which trikresol had been added, had been kept in a corked bottle, exposed to diffused light, the temperature of the room being very high during the summer months. The first and second rabbits of the series treated with human pleuritic exudation, etc., received only one and two injections respectively of fresh serum, being treated for the rest of the time with pleuritic effusion which had been kept at room-temperature for *five to six months*. The pleuritic fluid had been preserved in a corked bottle, with chloroform. These observations seem to me to possess a particular interest.

It seemed of interest, from a medico-legal standpoint, to determine whether or no a *mixture* of two kinds of blood would prevent the detection of one of the bloods in the mixture, the presence of another blood might inhibit the action of the anti-serum. To determine this question 1 : 100 dilutions of two kinds of blood were mixed together in equal proportions and tested as shown in the following table.

Table showing the results of tests made with blood-mixtures.

Bloods mixed	Anti-serum used	Result		No reaction produced by non-homologous anti-sera or normal rabbit-serum	
Human and ox	Human*	Marked reaction		Horse* Dog Ox Sheep Dog	
" " sheep					
" " dog					
" " horse					
" " cat	"	"	"	Normal rabbit serum	
Ox and human	Ox	"	"		
" " dog	"	"	"	"	"
" " horse	"	"	"	"	"
Dog and human	Dog	"	"	"	"
" " horse	"	"	"	"	"
" " ox	"	"	"	"	"
Sheep and human	Sheep	"	"	"	"
" " dog	"	"	"	"	"
" " horse	"	"	"	"	"
Horse and human	Horse	"	"	"	"

* The names in this column indicate the blood with which the rabbit was treated which yielded the anti-serum.

Each blood in the mixtures included in the table was actually diluted to 1 : 200 by the addition of the equal volume of other blood dilution.

From a medico-legal standpoint, it seemed important to determine whether the reaction would take place in the presence of several other bloods. For this reason three different mixtures were made, as follows:

Mixture I. contained equal volumes of ox, sheep, horse, cat, and human blood dilutions (each about 1 : 100). When the anti-serum for human blood was added to this mixture, a marked reaction immediately took place, whereas no reaction followed when we added normal rabbit or cat serum, as also anti-serum for dog's blood.

Mixture II. contained equal volumes of human, dog, horse, sheep, ox, and newt blood dilutions (each about 1 : 100). Reactions were obtained on the addition of the anti-sera for human and dog blood, whereas both normal rabbit serum and cat serum produced no reaction.

Mixture III. contained equal volumes of dog, gazelle, swallow, wild rabbit, duck and snake blood dilutions (each about 1 : 100). A marked reaction was obtained on the addition of the anti-serum for dog's blood, whereas no reaction followed upon the addition of the anti-serum for human blood, as also upon the addition of cat serum, and the serum of a normal rabbit.

We see from these experiments that a blood can be detected even when mixed with that of several other animals, each blood in the mixture being diluted about 1 : 500 or 1 : 600.

CONCLUSIONS.

1. The investigations we have made confirm and extend the observations of others with regard to the formation of specific precipitins in the blood-serum of animals treated with various sera.

2. These precipitins are specific, although they may produce a slight reaction with the sera of allied animals.

3. The substance in serum which brings about the formation of a precipitin, as also the precipitin itself, are remarkably stable bodies.

4. The new test can be successfully applied to a blood which has been mixed with that of several other animals.

5. We have in this test the most delicate means hitherto discovered of detecting and differentiating bloods, and consequently we may hope that it will be put to forensic use.

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THE USE OF THE GRAPHIC METHOD IN TRACING THE
DISTRIBUTION OF MILK-CARRIED SCARLET FEVER
ILLUSTRATED BY AN OUTBREAK IN CLIFTON, IN
1900.

With Chart.

By D. S. DAVIES, M.D.,
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THE Graphic Method, which I first used in connection with the outbreak of milk-carried enteric fever¹ which occurred in Clifton in 1897, is here applied to a similar outbreak of milk-carried scarlet fever.

The dairy-farm X is regarded as the source of infection. On this farm at the time of the outbreak a boy who had access to the milk vessels was suffering from an unrecognized illness which was compatible with a mild (ambulant) attack of scarlet fever, and within a week two brothers sickened with well-marked scarlet fever. The veterinary surgeon found no evidence of disease amongst the cows, and no other persons connected with the dairy-farm showed signs of illness.

The dairy-farm X supplied milk to two Clifton distributors Y and Z, and also distributed milk in the city direct from the farm; no other retailers obtained any milk from X.

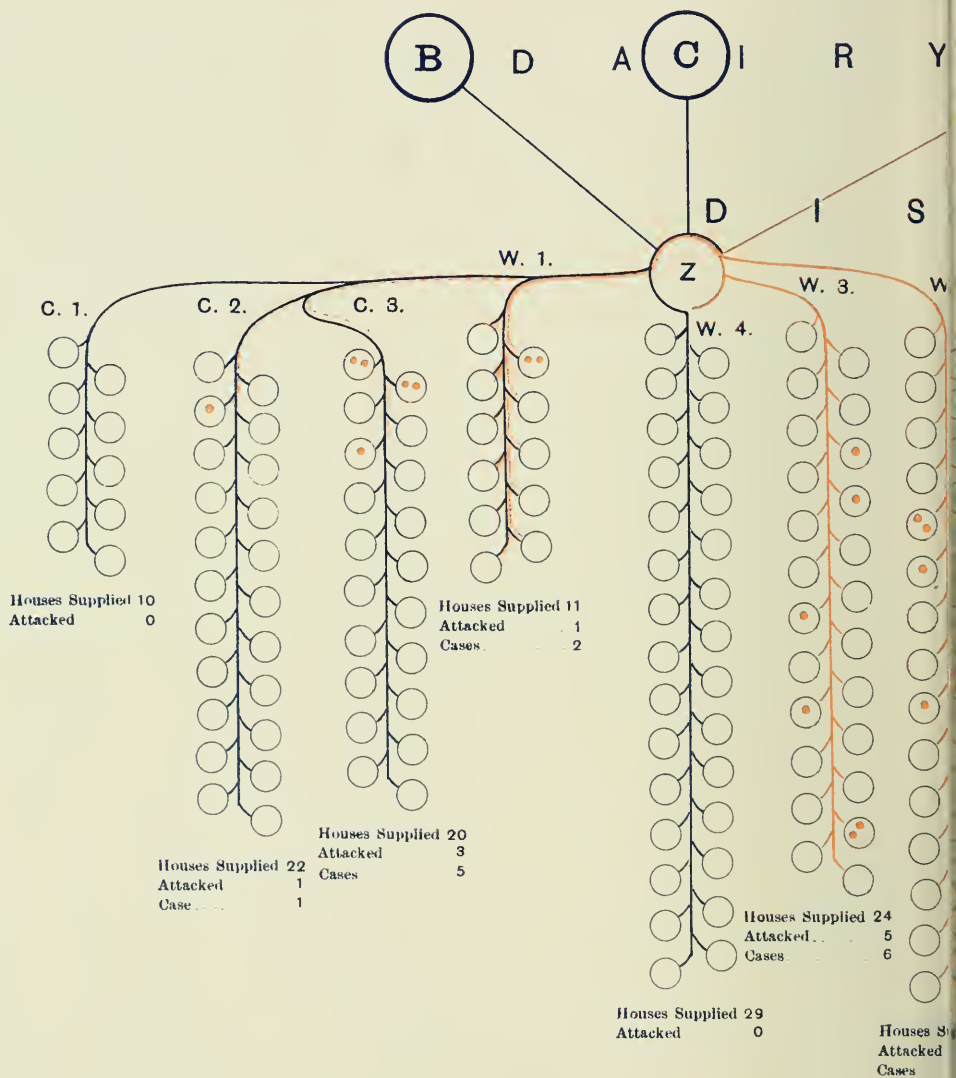
These three distributors receiving X milk supplied 269 houses, of which 42 were attacked, furnishing 66 cases; that is, one in every 6.4 houses was attacked. During the same period 85 other distributors not receiving X milk supplied 6922 houses; and 9 cases occurred in as many houses, that is, one in every 769 houses was attacked, an incidence not indicating any unusual prevalence of scarlet fever in a large city. (Clifton has a population of 47,301. It is a registration sub-district of Bristol, which has a population of 324,973.)

Y obtained a part of his milk from a dairy-farm A, but as A also supplied the retailers D and E, whose rounds were absolutely free from scarlet fever, this farm is at once cleared from any suspicion.

¹ *Medico-Chirurg. Trans. of the Royal Med. and Chirurg. Soc. of London*, Vol. LXXXI. 1898, p. 125. *Transactions of the Epidemiological Society of London*, Vol. XVII. Session 1897-98, p. 78.

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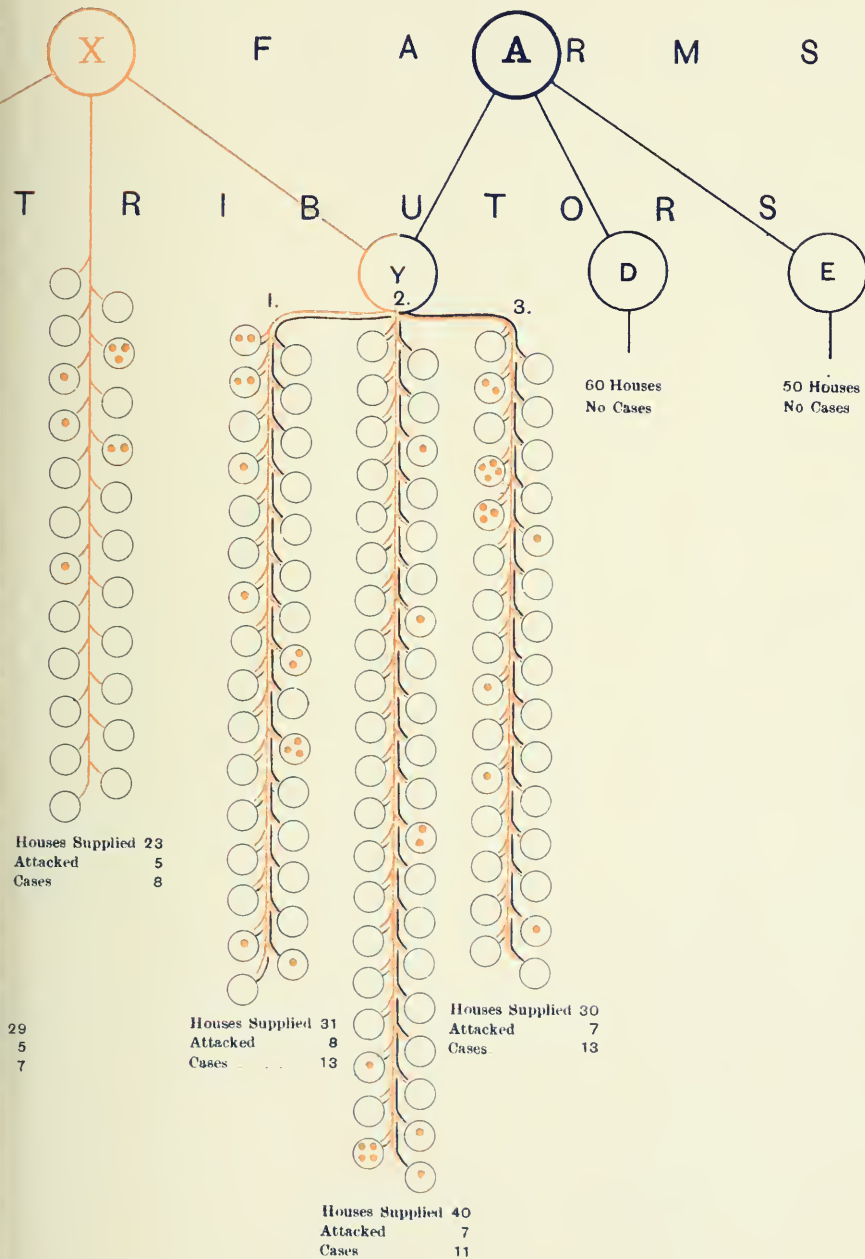
Illustrating the Graphic Method of tracing the outbreak
applied to an Outbreak



Three Distributors supplying 269 houses.
42 houses attacked.—One house in every 6.4.

R T

Distribution of Milk-carried Scarlet Fever
in Clifton, 1901.



85 Other Milk Distributors supplied 6922 houses.
9 houses attacked.—One house in every 769.

390 *Graphic Method in Milk-carried Scarlet Fever*

The immunity of W 4 street seemed at first remarkable, as it was on the supply from X churn, but as this street is invariably dealt with last, and as the infection probably occurred upon a single occasion, all the cases sickening within a few days, absolute exemption of W 4 street was quite likely to occur in the circumstance of the X supply running short on that particular day, which the distributor Z believes may have happened on or about Saturday or Sunday, the 13th or 14th October.

The W 1 round was amongst a different class of houses limited in number, and admittedly supplied on occasion from B; whereas W 2 and W 3 streets got no chance of exemption from the X supply, and suffered heavily.

The other general rounds (C_1 , C_2 , C_3) of this distributor in Clifton were as a matter of routine practice restricted to the milk supplied from farms B and C, but in order to make up quantity small portions of X milk were occasionally added.

Houses supplied	52	} 7.6%	Cases 6.
„ attacked	4		

This milk was sent out on three district rounds, one of which (C_1) remained exempt, the second had 1 case, the third showed 3 infected houses and 5 cases.

THE GREAT BACTERIAL CONTAMINATION OF THE MILK OF CITIES. CAN IT BE LESSENER BY THE ACTION OF HEALTH AUTHORITIES?

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It will be accepted that milk containing large numbers of bacteria, as well as the products of their growth, is less suitable for food than unpolluted milk. A bacteriological examination of the milk in great cities¹ generally will show that much of it in hot weather, and some of it at all seasons of the year, abounds in bacteria. Clinical experience also teaches that much of the milk in hot weather is unsuitable for food, especially for infants; because in them, owing to the rapidity with which the milk passes through the stomach, gastric digestion is almost no safeguard against the entrance of disturbing microorganisms into the intestines. Even pasteurization of milk charged with bacteria and their products does not restore it to its original condition, for the dead bodies of the bacteria and their toxins still remain. The changes in milk which are most deleterious being now known to be due to bacteria, it is theoretically conceded by all, that commercial cow's milk, the substitute for maternal milk, should be as nearly free as practicable from bacteria.

This being so, it seems strange that those who care for the health of communities, while striving so earnestly to protect milk from chemical adulteration, do nothing to compel or even encourage the farmer and shipper to supply milk free from excessive numbers of bacteria and their toxins. This neglect is probably due to the fact that Health

¹ According to Pakes, London milk contains over 3,000,000 bacteria per c.c. (*Lancet*, Feb. 3, 1900.)

Authorities have considered the practical difficulties too great to be overcome.

The investigations, the results of which are here given, were undertaken with the object of determining how much contamination necessarily takes place in milk during milking, and how much bacterial growth afterwards in its transit from the dairy to the city. The results show that it is perfectly practicable for the farmer and shipper, without appreciable increase of expense, to furnish commercial cow's milk with far less bacterial contamination than that now supplied.

From a careful study of the question, I think that the milk now consumed is unnecessarily contaminated and unwholesome, and that this is largely due to the present almost complete ignorance of persons, commercially interested, who do not appreciate the fact that bacteria arising from contamination by stable and barnyard dirt are capable, unless inhibited by cold (with the present allowance of time for transportation), of an enormous development which may render good milk utterly unfit for food. Although this study applies to the conditions existing in one city only, I hope that the facts brought out may be of general use.

The Source of New York Milk.

Nearly all the New York milk is transported to the city by railroads, most of it travelling a distance of 50 to 350 miles. The milk is delivered in the city about 2 a.m., consequently when it is shipped at a point distant 350 miles from New York it must start on its journey as early as 8 a.m. on the previous day. Under existing conditions the milk which reaches New York must, therefore, have been kept from 12 to 36 hours. Twelve hours more must elapse before the last of the milk is used. The milk is usually delivered at New York in 40-quart cans, or in quart jars. The dealers in the city have no control over the extent of bacterial growth which exists in their milk, though they may prevent further contamination and exclude further development by the application of cold.

The problem in New York and other cities of great size is, therefore, to furnish milk in a condition suitable for food after it has been kept from 24 to 48 hours. Milk even staler than 48 hours is now sold, but this is totally unnecessary and should not be allowed.

The present dangerous condition of the milk of great cities, especially in hot weather, arises partly from the fact that the great growth of the

cities has so widened the area from which milk is obtained. The cleanliness, cooling, and interval of time consumed in transportation, which sufficed for milk to be drunk in the neighbourhood, are utterly insufficient for milk which is consumed at a distance of 300 miles from the place of milking. The number of bacteria present in the milk of healthy cows, depends almost entirely on four things: (1) The original amount of germicidal substance in the milk; (2) The amount of bacterial contamination on or after milking; (3) The length of time which has elapsed since milking; (4) The temperature at which the milk has been kept. We have no control over the germicidal qualities of milk; but we can insure cleanliness in procuring milk. The degree to which milk is cooled and the length of time it is kept, can also be almost completely controlled.

There is an inexcusable lack of cleanliness in the methods of procuring milk and of care in sufficiently cooling and keeping it during its transportation. Even in the matter of sending milk to the railroad many farmers take twenty-four hours more than is necessary, keeping back one-half of their milk in order to save the trouble and expense of making more than one trip each day to the station. In considering what can be done to improve the New York milk supply, we may first note the comparatively small number of bacteria in milk which has been obtained and kept under suitable conditions, then the moderate number in milk which has been procured under ordinary dirty conditions, but which has been properly cooled and quickly transported, and finally the large number in the milk sold in New York under present conditions.

These data are given in the following observations:

I.

The number of bacteria present at the time of milking and 24, 48 and 72 hours afterwards in milk obtained and kept under correct conditions.

No preservatives were present in any of the following specimens.

TABLE I.

Milk obtained where every reasonable means was taken to insure cleanliness. The long hairs on the udder were clipped; the cows roughly cleaned and placed in clean barns before milking; the udders were wiped off just previous to milking; the hands of the men were washed and dried; the pails used had small (six-inch) openings, and were thoroughly cleaned and sterilized by steam before use. Milk cooled within one hour after milking to 45° F., and subsequently kept at that temperature. The first six specimens were obtained from individual cows; the last six from mixed milk as it flowed at different times from the cooler. Temperature of barns 55° F.

*Number of Bacteria in 1 c.c. of Milk¹.**From six individual cows.*

5 hrs. after milking	After 24 hrs.	After 48 hrs.	After 72 hrs.
500	700	12,500	Not counted
700	700	29,400	" "
19,900	5,200	24,200	" "
400	200	8,600	" "
900	1,600	12,700	" "
13,600	3,200	19,500	" "
Average 6,000	1,933	17,816	

From mixed milk of entire herd.

6,900	12,000	19,800	494,000
6,100	2,200	10,200	550,000
4,100	700	7,900	361,000
1,200	400	7,100	355,000
6,000	900	9,800	445,000
1,700	400	8,700	389,000
Average 4,333	2,766	10,583	329,000

Twenty-five samples taken separately from individual cows on another day and tested immediately averaged 4,550 bacteria per c.c. and 4,500 after 24 hours. These 25 specimens were kept at between 45° and 50° F.

II.

Milk taken during winter in well ventilated, fairly clean, but dusty barns. Visible dirt was cleaned off the hair about the udder before milking. Milker's hands were wiped off but not washed. Milk pails and cans were clean, but the straining cloths dusty. Milk cooled within two hours after milking to 45° F.

TABLE II.

Number of Bacteria in 1 c.c. of Milk.

At time of milking	After 24 hrs.	After 48 hrs.
12,000	14,000	57,000
13,000	20,000	65,000
21,500	31,000	106,000
Average 15,500	21,666	76,000

¹ Number of bacteria obtained from development of colonies in nutrient agar in Petri plates. The nutrient medium contained 2% peptone and 1.2% agar, and was faintly alkaline to litmus. One set of plates were usually left four days at about 20° C. and one set 24 hours at 37° C., and then 24 hours at 20° C. From 5 to 30% more colonies developed as a rule in the plates kept at room-temperature than in those kept for 24 hours at 37° C. The milk was diluted as desired with 100 or 10,000 parts of sterile water, and 1 c.c. of the diluted milk was added to 8 c.c. of melted nutrient agar. Plates containing over 1,000 colonies were found to be inaccurate, in that they gave too low totals. Apparently a considerable number of bacteria failed to develop colonies when too many were added to the nutrient agar. Nutrient gelatine was found to be more troublesome and not to yield more accurate results than nutrient agar.

III.

Milk taken from cows in ordinary barns. Ground covered thick with manure, the cows being more or less visibly dirty. The teats were cleaned slightly by running the unwashed hands over them once before milking. Pails and cans were thoroughly cleaned but not sterilized by heat. Milk cooled to 45° F. within two hours after milking.

TABLE III.

Bacteria in 1 c.c. of Milk.

Shortly after milking in warm weather	Shortly after milking in winter weather
18,300	11,500
18,300	11,600
21,200	17,800
22,000	18,900
51,200	19,900
51,200	20,200
Average 30,366	16,650
Average after 24 hrs. 48,000.	Average after 24 hrs. 31,000.
„ „ 48 hrs. 680,000.	„ „ 48 hrs. 210,000.

IV.

The condition of the average city milk is very different and is shown in the following tables.

The twenty samples were taken late in March by Inspectors of the Department of Health of New York City from cans of milk immediately upon their arrival in the city.

The temperature of the atmosphere averaged 50° F. during the previous 24 hours. The temperature of the milk when taken from the cans averaged 45° F. Much of this milk had been carried over 200 miles. From the time of its removal from the cans, which was at about 2 a.m., until its dilution in nutrient agar at 10 a.m. the milk was kept at about 45° F.

TABLE IV.

*From New York and Hudson River
Railroad.*

No. of sample	No. of bacteria in 1 c.c.
50	35,200,000
51	13,000,000
52	2,500,000
53	1,400,000
54	200,000
55	600,000
56	2,500,000
57	100,000
58	3,700,000
59	135,000
Average per c.c.	5,933,500

From Harlem Railroad.

No. of sample	No. of bacteria in 1 c.c.
48	6,200,000
49	2,200,000
50	15,000,000
51	70,000
52	80,000
53	320,000
54	5,000,000
55	140,000
56	25,000,000
57	52,000
Average per c.c.	5,406,200

It is interesting to note that while the average number of bacteria in the samples is very high, yet in nearly one-half of the specimens the number is low. The high figures were obtained from improperly handled milk.

V.

Milk as sold in the shops during the morning hours gave the following results :—

TABLE V.

Column *A* shows the number of bacteria per c.c. in 13 samples of milk supplied to the poorer tenement districts in midwinter. Average temperature of the milk 41° F. (highest 46°, lowest 38°).

Column *B* shows the corresponding number of bacteria in 10 samples taken from different dairy stores throughout the portion of the city inhabited by the more "well-to-do" classes in midwinter.

Column *C* gives corresponding figures for 5 samples from tenement districts early in September. Average temperature of the air during the preceding 24 hours, 78° F. Average temperature of samples 50° F.

Column *D* contains the results obtained early in September from milk sold in the "well-to-do" districts.

Number of Bacteria in 1 c.c. of Milk.

<i>A</i>	<i>B</i>	<i>C</i>	<i>D</i>
110,000	30,000	5,600,000	80,000
140,000	30,000	6,100,000	192,000
140,000	60,000	15,910,000	355,000
145,000	60,000	16,320,000	480,000
175,000	70,000	31,888,000	4,200,000
280,000	155,000		
320,000	240,000		
560,000	560,000		
640,000	650,000		
1,200,000	1,420,000		
6,400,000			
5,100,000			
10,500,000			
Average 1,977,692	327,500	Average 15,163,600	1,061,400

While the above figures indicate that much of the milk sold in the better class stores is fair, even in summer, they show an appalling condition for most of that sold to the poorer classes—those who not only comprise the larger part of the population, but who are also compelled to keep their children in town during the hot weather.

It must be kept in mind that milk averaging 13,000,000 bacteria per c.c. will, when kept at the temperature common in the homes of the poor, soon contain very largely increased numbers and show its dangerous condition by turning sour and curdling.

A study of the present methods of handling milk makes it clear why so much of the milk contains excessive numbers of bacteria, and also fortunately how a great improvement can be effected by a few simple changes in the methods of handling milk.

*The Influence of Temperature upon the Rapidity of Bacterial
Multiplication in Milk.*

Few even of the well informed appreciate how great a difference a few degrees of temperature will make in the rate of bacterial multiplication. Milk rapidly and sufficiently cooled keeps almost unaltered for 36 hours, while milk insufficiently cooled deteriorates rapidly.

The majority of the bacteria met with in milk grow best at temperatures above 70° F., but they also multiply slowly even at 40° F.; thus of 60 species isolated by us, 42 developed good growths at the end of 7 days at 39° F. Our observations have shown that the bacteria slowly increase in numbers after the germicidal properties of the milk have disappeared, and the germs have become accustomed to the low temperature. In fact milk cannot be permanently preserved unaltered unless kept at 32° F. or less. The degree of cooling to which ordinary supplies of milk are subjected, differs greatly in various localities. Some farmers chill their milk rapidly, by means of pipe-coils over which the milk flows; others use deep wooden tanks filled with water into which the cans of milk are placed soon after milking. In winter these methods are very satisfactory, for the water runs into the pipes or tanks at about 38° F. In warmer weather they are unsatisfactory, unless ice is used, as the natural temperature of the water may be as high as 55° F. A considerable quantity of milk is not cooled at all at the farms. It is sent to the creamery or railroad after 2 to 6 hours, and is then more or less cooled. These few hours in summer, when the milk is left almost at blood heat, allow an enormous development of bacteria to take place as is shown in the table below.

TABLE VI.

Showing the development of bacteria in two samples of milk maintained at different temperatures for 24, 48 and 96 hours, respectively. The first sample of milk was obtained under the best conditions possible, the second in the usual way. When received, specimen No. 1 contained 3,000 bacteria per c.c., specimen No. 2 30,000 per c.c.

Temperature. Fahrenheit	Time which elapsed before making test			
	24 hrs.	48 hrs.	96 hrs.	168 hrs.
32°	2,400	2,100	1,850	1,400
	30,000 *	27,000	24,000	19,000
39°	2,500	3,600	218,000	4,200,000
	38,000	56,000	4,300,000	38,000,000
42°	2,600	3,600	500,000	
	43,000	210,000	5,760,000	
46°	3,100	12,000	1,480,000	
	42,000	360,000	12,200,000	
50°	11,600	540,000		
	89,000	1,940,000		
55°	18,800	3,400,000		
	187,000	38,000,000		
60°	180,000	28,000,000		
	900,000	168,000,000		
68°	450,000	25,000,000,000		
	4,000,000	25,000,000,000		
86°	1,400,000,000			
	14,000,000,000			
94°	25,000,000,000			
	25,000,000,000			

* The figures referring to tests of the second sample are printed in italics.

Observations on Bacterial Multiplication in Milk at 90° F., a temperature common in New York in Hot Summer Weather.

TABLE VII.

Number of Bacteria per 1 c.c.

	Milk I Fresh and of good quality	Milk II Fair quality from store	Milk III Bad quality from store
Original number	5,200	92,000	2,600,000
After 2 hrs.	8,400	184,000	4,220,000
„ 4 „	12,400	470,000	19,000,000
„ 6 „	68,500	1,260,000	39,000,000
„ 8 „	654,000	6,800,000	124,000,000

A sample of milk No. I. removed after 6 hours and cooled to 50° F. contained 145,000,000 at the end of 24 hours. Some of this milk which was kept cool from the beginning, contained but 12,800 bacteria per c.c. at the end of 24 hours.

*Time Required for the Transportation of Milk from the
Farm to the City.*

On inspection of dairy-farms it is found that farmers make but slight attempts to hurry their milk to market, except perhaps in the very hottest weather.

Thus, if the milk-train leaves a town daily at 9 p.m. the farmer who finishes milking at 5 p.m., and cooling the milk by 6 p.m., does not send the milk to the station that evening, but waits until the next day, so that he can send the milk from the morning's milking at the same time, and save the trouble of an extra trip. Thus, instead of one-half of his milk being delivered at the city after 12 hours, 36 hours will have elapsed before it reaches the consumer. The same is true of farmers living at a greater distance from the city, who instead of getting their milk to the city after an interval of 24 and 36 hours, get it there after 48 and 36 hours, making it 60 and 48 hours old when it reaches the consumer. As the milk is usually only cooled to about 52° F. the immense development of bacteria permitted through this unnecessary addition of 24 hours to the shipping time is apparent. A glance at the previous table, as well as at the following figures, illustrates this.

*Number of Bacteria present in Milk taken from cows in common dirty stalls,
24, 36 and 48 hours after milking. Milk cooled only to 52° F. three
hours after milking and maintained at that Temperature.*

TABLE VIII.

<i>Number of Bacteria per 1 c.c. of Milk.</i>				
After	3 hrs.	24 hrs.	36 hrs.*	48 hrs.
	21,200	70,000	350,000	1,600,000
	51,200	64,600	333,000	1,250,000
	18,300	61,000	305,000	1,400,000
	22,000	76,000	380,000	2,300,000
	51,200	64,000	320,000	1,280,000
	18,300	81,000	405,000	2,180,000
Average	30,366	69,433	348,833	1,668,333

* These figures at 36 hours are estimated from the test of one sample only.

By simply compelling the farmers within a radius of 100 miles from the city to send their afternoon's milk on the train of that evening, and those further off to send the morning's milk on the

morning train, a great decrease in the amount of bacteria would be obtained, since no milk would be delivered at a later time than 36 hours after milking.

The Degree of Cleanliness used in obtaining Milk and its Influence.

The present conditions under which much of the milk is obtained are not pleasant to consider. In winter, and to a less extent at other seasons of the year, the cows in many stables stand or lie down in stalls in the rear portion of which there is from one to four inches of manure and urine. When milked the hands of the milkers are not cleansed, nor are the under portions of the cows, only visible masses of manure adhering to the hair about the udder being removed. Some milkers even moisten their hands with milk, to lessen friction, and thus wash off the dirt of their hands and of the cow's teats into the milk in the pails. Some may regard it as an unnecessary refinement to ask that farmers should roughly clean the floors of their stalls once each day, that no sweeping should be done just before milking, and that the udders should be wiped with a clean damp cloth and the milkers should thoroughly wash and wipe their hands before commencing milking. The pails and cans should not only be carefully cleansed but afterwards scalded out with boiling water. The washing of the hands would lessen the number of ordinary filth bacteria in the milk, and diminish risk of transmitting to milk human infectious diseases like scarlet fever, diphtheria, and enteric fever, by the direct washing off of the disease germs from infected hands. It would also inculcate general ideas of the necessity of cleanliness and of the danger of transmitting disease through milk. The value of cleanliness in limiting the number of bacteria is demonstrated by the figures contained in the tables.

Summary and Conclusions.

Because of its location and its hairy covering the cow's udder is always more or less soiled with dirt and manure unless cleaned. On account of the position of the pail and the access of dust-laden air it is impossible to obtain milk by the usual methods without mingling with it a considerable number of bacteria. With suitable cleanliness, however, the number is far less than when filthy methods are used, there being no reason why fresh milk should contain in each c.c. on the

average, more than 12,000 bacteria in warm weather and 5,000 in cold weather. Such milk, if quickly cooled to 46° F., and kept at that temperature, will at the end of 36 hours contain on the average less than 50,000 bacteria per c.c., and if cooled to 40° F. will average less than its original number.

With only moderate cleanliness such as can be employed by any farmer without adding appreciably to his expense, namely, clean pails, straining cloths, cans or bottles, and hands, a fairly clean place for milking, and a decent condition of the cow's udder and the adjacent belly, milk when first drawn will not average in hot weather over 30,000, and in cold weather not over 25,000 bacteria per c.c. Such milk if cooled to and kept at 50° F., will not contain at the end of 24 hours over 100,000 bacteria per c.c. If kept at 40° F. the number of bacteria will not be over 100,000 after 48 hours.

If, however, the hands, cattle and barns are filthy, and the pails are not clean, the milk obtained under these conditions will, when taken from the pail, contain very large numbers of bacteria, even up to a million or more per c.c.

Freshly drawn milk contains a slight and variable amount of bactericidal substances which are capable of inhibiting bacterial growth. At temperatures under 50° F. these substances act efficiently unless the milk is filthy for from 12 to 24 hours, but at higher temperatures their effect is very soon completely exhausted, and the bacteria in such milk will then rapidly increase. Thus the bacteria in fresh milk which originally numbered 5,000 per c.c. decreased to 2,400 in the portion kept at 42° F. for 24 hours, but rose to 7,000 in that kept at 50° F., to 280,000 in that kept at 65° F., and to 12,500,000,000 in the portion kept at 95° F.

As we have seen, the milk in New York City is found on bacteriological examination to contain as a rule excessive numbers of bacteria. During the coldest weather the milk in the shops averages over 300,000 bacteria per c.c., during cool weather about 1,000,000, and during hot weather about 5,000,000. The milk in other large cities is from all accounts in about the same condition.

The above statement holds for milk sold at the ordinary shops, and not that of the best of the special dairies, where, as previously stated, the milk contains only from 10,000 to 30,000 bacteria according to the season of the year.

The question might be raised, Are even these enormous numbers of bacteria in milk during hot weather actually harmful? Here we

have only to refer to universal clinical experience, that a great number of children in cities sicken on the milk supplied in summer, that those put on milk which is sterile or contains few bacteria as a rule mend rapidly, while those kept on the impure milk continue ill or die.

Our knowledge is probably as yet insufficient to state just how many bacteria must accumulate to make them noticeably dangerous in milk. Some varieties are undoubtedly more harmful than others and we have no way of restricting the kinds that will fall into milk, except by enforcing cleanliness. We have also to consider that milk is not entirely used for some twelve hours after being purchased, and that during all this time bacteria are rapidly multiplying, especially where, as among the poor, no provision for cooling it is made. Slight changes in the milk which to one child would be harmless, would in another produce disturbances which might lead to serious disease. A safe conclusion is that no more bacterial contamination should be allowed than it is practical to avoid. Any intelligent farmer can use sufficient cleanliness and apply sufficient cold, with almost no increase in expense, to supply milk 24 to 36 hours old which will not contain in each c.c. over 50,000 to 100,000 bacteria, and no milk containing more bacteria should be sold.

The most deleterious changes which occur in milk during its transportation are now known not to be due to skimming off the cream, or to the addition of water, but to the changes produced in the milk by multiplication of bacteria. During this multiplication, acids, and distinctly poisonous bacterial products are added to the milk, to such an extent that much of it has become distinctly deleterious to infants and invalids. It is the duty of Health Authorities to prevent the sale of milk rendered unfit for use through excessive numbers of bacteria and their products.

The culture-tests to determine the number of bacteria present in any sample of milk require at least 48 hours, so that the sale of milk found impure cannot be prevented. It will, however, be the purpose of the authorities gradually to force the farmers and the middlemen to use cleanliness, cold and despatch in the handling of their milk, rather than to prevent the use of the small amount tested on any one day.

If the milk on the train or at the dealer's were found to contain excessive numbers of bacteria, the farmers would be cautioned and instructed to carry out the simple necessary rules, which would be furnished. If they failed to correct the evil, the Health Authorities

would, by refusing permits to the sellers, or in other ways, prevent the further sale of such milk. Thus the present lack of interest of dealers and farmers regarding the bacterial purity of their milk would be overcome.

If the authorities decide to establish a standard of bacterial purity for milk, what should it be?

We must recognize that much of the present impurity of milk in hot weather is due to the ignorance of the farmers and carriers, also that it will be well, if possible, to have their cordial co-operation in bettering the quality of the milk. It seems to the writer, therefore, that at first it would be more important to establish the principle that excessive bacterial multiplication in milk is harmful, and to get the co-operation of all those who deal with milk to do their best to limit this multiplication, than to fix any definite number of bacteria as the limit above which milk must be destroyed. Some figures, however, must be adopted by the authorities, even at first, beyond which milk cannot be allowed to be distributed. For the first year I think 500,000 per c.c. for milk entering New York and 1,000,000 per c.c. for milk delivered to the consumer might be a practical standard. If no milk worse than the above was brought in or distributed there would be a vast improvement over previous years, and, as a matter of fact, no dealer could afford to try and approach the limit, for if he did he would frequently go beyond it. Thus even by enforcing a standard allowing at least tenfold the number of bacteria which should be present in milk, a great improvement in our milk supply and a compulsory education of the farmer in the need of cleanliness, the preservative effect of cold, and a few of the elementary laws governing the transmission, the multiplication and the products of bacteria, would be secured. The difficulties which would be met with in distributing knowledge, in carrying out the tests, and in enforcing better methods would undoubtedly be great in cities of the size of London and New York: but the size of a city increases the need even more than the labour of the work; and wholesome, clean, unfermented milk is certainly of sufficient importance to make it worth while to undertake far more difficult tasks than this will prove to be.

On May 8th, 1901, the Department of Health of New York City adopted measures to prevent, after a reasonable time, the introduction into New York City of milk which contains unnecessary numbers of bacteria. It will be of great interest to watch the results of this action.

APPENDIX.

CIRCULAR OF INFORMATION FOR FARMERS RELATING TO
THE COLLECTION AND CARE OF MILK¹.*Issued by the Department of Health of New York City.*

The Department of Health of New York City has determined to adopt stringent measures against the introduction into New York of milk which contains an unnecessary and dangerous number of germs or bacteria.

The investigations of the Department have shown that under proper conditions and with reasonable care milk reaches the city in excellent condition, containing but a comparatively small number of germs. Where large numbers of germs are present in it, experience has shown that it is always the result of an unusual lack of cleanliness, or some serious defect in the methods of collection, handling, or care of the milk.

This circular is issued by the Department for the information of farmers and dairymen so that they may in their own interests observe those precautions which are necessary to preserve the milk in good condition and thus prevent its being condemned.

DIRECTIONS.

1st. The greater the cleanliness observed in collecting milk, the smaller will be the number of germs which drop into it.

2nd. The quicker the temperature of the milk is reduced and the lower it is kept the slower the growth of bacteria in it will be.

3rd. The quicker the milk is transported to the consumer the less time there is for the multiplication of germs and the better will be the condition of the milk when delivered.

*A Detailed Consideration of these three Factors which influence
the Condition of Milk.*

1st. **The Barns.** The barns should be kept clean, so that the cattle will not become filthy from lying in manure. If the cows are milked in the barns, no sweeping should be done a short time before milking, otherwise the dust raised, which is full of germs, will settle into the milk. If possible, a separate clean shed should be used for milking. The barns and the dairy building, if there is one, should be some distance from the dwelling house, to limit the danger of transmitting through the milk any contagious disease which may occur among the inmates of the house. Barns should be well ventilated and dry, so as to keep the cattle healthy.

2nd. **The Water.** The water used for cleaning the pails, cans, and for all other purposes in connection with the milk should be from a source at some

¹ This circular is to be distributed to all farmers who send milk to New York City.

distance from the house and barn, so that there shall be no danger of pollution by sewage.

3rd. The Cows. The majority of the germs which enter milk come from dirt which is shaken from the cow's udder and belly during milking. In order to have clean milk, it is necessary to prevent this; therefore previous to milking, the udder and the adjacent belly should be cleaned of dirt. All visible manure from these portions and from the tail should be removed, and the udder and the skin of the belly surrounding it should be wiped with a damp cloth. This largely prevents it from being shaken off during milking.

The milk should not be used from cows whose udders are diseased, or who are themselves unhealthy in any way.

4th. The Milkmen. All who come in contact with the cows or the milk should be free from contagious disease, and should not come in contact with any case of contagious disease, such as diphtheria, scarlet fever or measles. These and other diseases may be transmitted by the infection of the milk.

Before milking, the milkmen should thoroughly wash their hands with clean soap and water and dry them on a clean cloth, in order to remove all dirt and germs from them. This should be done after the cow's udder, belly and tail have been cleaned. Milkmen should never moisten their hands with milk, in order to lessen the friction in milking, as this tends to cause dirty milk to drip into the milk-pail.

5th. The Pails, Cans, Straining Sieves and Cloths. These should all be absolutely clean. They are very frequent sources of extensive contamination of the milk. They should all be cleaned immediately after use, first with lukewarm water, and then sterilized by being scalded with boiling water. After being thoroughly washed they should be placed upside down, to prevent dust falling into them, as this contains great numbers of germs. Straining sieves and cloths should be covered over to protect them from dust.

6th. Cooling. Milk should be cooled quickly to 45° F., or less. The simplest way is by placing the cans in a large wooden tank containing cold water. Except in winter, the water should be cooled and kept cold by the addition of ice, so as to be at 40° F., or less, and the cans should be immersed up to their necks and left to stand at least one hour, or until shipped to the creamery or train. If any milk in the cans stands at a level higher than the surrounding water, it is scarcely cooled at all for many hours.

The milk having been obtained in a cleanly manner and quickly cooled, should be delivered cold at the creamery or train. A full can of milk retains its cold for some time, but in summer should not be exposed to the warmth of the air for over one hour. If it is to be kept for a longer period at the station, some arrangements for keeping it cool must be made. Milk should never be allowed to stand in the sun.

7th. Transportation. Milk should be shipped to the city as quickly as possible. Many farmers hold the evening's or morning's milk over an entire day, to avoid the inconvenience of early delivery. This causes great injury to the milk. Dairymen have come to believe that as long as the milk is delivered in the city in such condition that it will remain sweet until delivered to the consumer, nothing more is required. Such milk when sold often contains enormous numbers of germs, is unwholesome, dangerous, and capable of causing much sickness and death, especially

in the summer. The high death-rate from diarrhoea among infants in the summer in the city is wholly due to such milk. Milk known to be over 36 hours old, or containing large and unnecessary numbers of germs, will not in future be allowed to enter New York.

The Transmission of Contagious Diseases through Milk.

No farmer or dairyman should allow any one who has a contagious disease, or who has been in contact with any person having scarlet fever, typhoid fever, measles, diphtheria, or consumption, to have access to the cattle, or to have any connection with the milk or milking, or with the milk utensils. Epidemics and outbreaks of contagious disease are often produced through the infection of the milk in this way, and if cases of disease in New York are traced to any dairyman, and proof is found that disease has been transmitted through negligence on his part, the Department of Health will take summary action in relation to this.

THE SEASONAL PREVALENCE OF ANOPHELES AND
MALARIAL FEVER IN LOWER BENGAL; AND THE
PRACTICAL APPLICATION OF THE MOSQUITO
THEORY. (One Chart.)

By LEONARD ROGERS, M.D., M.R.C.P., I.M.S.

THE momentous discovery of the part played by *Anopheles* in the propagation of malaria necessitates careful local inquiries into the prevalence of this genus, in order to ascertain how far the different methods of lessening the amount of malaria which have recently been proposed can be profitably applied to the circumstances met with. In no country are such studies more essential than in India on account of the radical differences in the topographical and climatic conditions of different provinces of this vast and densely populated country, ranging from the waterless deserts of Sind to the steamy, waterlogged marshes of Lower Bengal. During the past year I have systematically studied this question in the last-named province, which is a home of malaria, where it is prevalent at all times of the year, and which presents most exceptional difficulties in the way of preventive measures. During the last few months I have travelled many hundred miles as Deputy Sanitary Commissioner, so have had exceptional opportunities of studying the problem, and now desire to put my conclusions on record, especially as some of the facts are not altogether easy to explain on the exclusive mosquito theory, although the experiences of others may possibly throw some light on them. The present inquiry was commenced at the beginning of 1900, when, although it was shown that malaria could be carried by mosquitos, it was not so certain that this is the only way in which the disease could be produced, as now seems likely to prove to be the case. At one time I was inclined to attribute considerable importance to the great divergence between the maximum seasonal prevalence of *Anopheles* (chiefly *A. rossii*) and of malarial

fevers; especially in view of the diminished incidence of malarial fever, as judged by the percentage of enlarged spleens, in areas supplied with filtered water, as compared with similarly situated areas which were without filtered water, to which I have elsewhere drawn attention⁽¹⁾. However, in view of the accumulation of evidence during the past year in favour of the mosquito theory, due allowance for defects in our knowledge of the life-history of different varieties of mosquitos must be made before any facts which appear to be opposed to the new theory are allowed any great weight, and those about to be recorded may be more easily understood as our knowledge of these insects increases.

The Physical Characters of the Area investigated.

The great difficulty in the practical application of the new knowledge to lessening malaria in Lower Bengal can only be appreciated when the nature of the country is understood. The vast area comprised in the deltas of the Ganges and Brahmaputra rivers, with its millions of inhabitants, is so low-lying that a great part of it is under water during the rainy season, when the numerous villages placed on any slightly elevated spots, often on the high banks of the network of small rivers, can only be reached by boat, while between them stretch interminable rice-fields, forming after the subsidence of the floods innumerable pools which it is quite impossible to either drain or treat with culicides. The freer distribution of quinine is the only practical remedy here. Unfortunately the conditions in small towns are only slightly more favourable on account of the innumerable tanks and smaller excavations in which they abound, due to the fact that all the houses are constructed on raised earth platforms, which can only be made by excavating the earth from the surrounding level ground. Thus it comes about that a town of some 3000 inhabitants will as a rule have over 100 tanks, and not infrequently a still larger proportion, in addition to a very much larger number of smaller excavations. Nor is it possible to fill up these tanks without digging others to supply the earth. The importance of this fact lies in the discovery which I made last year, that, contrary to even the recent statements of Major Ross⁽²⁾, tanks form a most important breeding place for *Anopheles*, and are indeed the most important one during the greater part of the year in the towns of Lower Bengal, as I shall show presently. This fact having been early discovered, an area of one-sixteenth of a square mile, containing 32 tanks and several

smaller pools and drains, was selected for regular examination for a year in order to study the relationship of the variations in the number and distribution of the *Anopheles* larvae and the prevalence of malaria, as indicated by the number of cases of malarial fever treated at the dispensary which supplies the medical wants of this part of the town. The place chosen is a very small corner of Calcutta itself, and is low-lying and very waterlogged, although the percentage of cases of enlarged spleen was low, apparently on account of the water supply being filtered. The area may be regarded as fairly typical of Lower Bengal towns, but somewhat more unfavourably situated than the average, and much more so than the European quarter of Calcutta.

The Seasonal Prevalence of Anopheles and Malaria during the Year.

The results of the year's inquiry are shown in the accompanying chart, the upper curve representing the weekly number of cases of malarial fever treated at the local dispensary. Below this curve the rainfall is graphically shown, while above it the results of the monthly mosquito hunt are briefly inserted, and at the bottom of the chart the curves of the mean weekly maximum and minimum temperatures are given. The marked and steady rise of the fever rate three weeks after the onset of the rainy season early in June is very evident, as is the continuance of the fever at a high rate through the rains and the following two months up to the middle of December, with an abrupt fall during the latter half of that month. No less definite is the longer season of low fever prevalence, which lasts from the middle of December throughout the rest of the cold weather and the whole of the hot season and the first two or three weeks of the rains in June. In February 1901 there was a slight secondary rise, but this did not occur in the previous year, and is not a constant feature. The temporary fall in the third week in September was due to floods preventing people attending the dispensary. The returns of the number of cases treated in hospitals in many parts of Lower Bengal show the same seasonal curve of intermittent fevers, varying but slightly in different places. The exact weeks in which the main rises and falls occur vary with the onset and cessation of the rains. Thus an examination of charts of the fever rate, rainfall, and temperature variations over a series of years in an important suburb of Calcutta shows that when the rains set in early and end early the rise and fall of the fever curve is also proportionally early in both its rise and fall, and *vice versâ*, so that the curve of the accompanying

chart may be fairly taken as typical of Lower Bengal in general, and of the neighbourhood of Calcutta in particular. Another point which must be mentioned here, as it will be alluded to again, is that, in the districts round Calcutta at any rate, the fever curve always rises with each break of a week or more in the rains during the monsoon, and the unhealthy years are those in which such breaks are frequent, quite irrespectively of the total amount of the rainfall.

Turning now to the variations in the prevalence of the *Anopheles* larvae, from which the adult insects must necessarily be derived, we are at once struck by the fact that their maximum prevalence is in the hot weather months of from March to May, when they are present in enormous numbers in the tanks, the climax being reached in the last-named month, when no fewer than two-thirds of the tanks were found to be infested, and in several of them, including one measuring 350 yds. by 70, the leeward end was covered with a scum of many million larvae and pupae in all stages of development. On the other hand, all the small pools and drains were dried up during this season with the exception of three in March. Yet these three hot weather months are precisely those in which malarial fevers are at their minimum, and in which those few cases which are met with are found to be of the chronic relapsing variety. With the onset of the rains in the middle of June *Anopheles* larvae were much less readily found, only being detected in one-sixth of the tanks examined during this month. At first I thought that this might be due to their being scattered by the rain, but searches after a few days' dry weather showed them to be equally scanty. Another change was the filling up of some previously dried-up pools, in four out of seven of which the *Anopheles* larvae were found; but the net result was a great decrease of the total number of larvae in this area, the diminution in the tanks being very many times as great as the slight increase in the few small pools. In July the conditions just mentioned were accentuated, the larvae being only found twice in a large number of examinations of the tanks, even during a marked break in the rains; while on the other hand on the 22nd of this month out of 14 pools, which had by this time filled with water, 10 contained the larvae, as did some roadside drains. Still the total number was very much smaller than in the hot weather months, as all the small pools put together did not contain a tithe of the numbers formerly met with in a single tank. On the other hand, owing more particularly to their presence in the roadside drains, the larvae were somewhat more widely distributed during the rainy season than in

the hot weather. Still as none of the thickly placed houses of the area under investigation were as much as fifty yards from an infected tank this factor cannot have been a very important one.

In this month a break in the rains occurred, advantage of which was taken to see if the cause of the increase of fever which had been noticed to closely follow such an event could be traced, but it was found that after four days out of the fourteen pools which had been found to contain the larvae, all in a very young stage, no less than nine had already dried up, and in only three were larvae still found. It is evident, then, that a break in the rains greatly diminished the already small number of larvae which were to be found during the feverish rainy season, and the increased fever during breaks in the rains cannot be explained by any corresponding increase of the numbers of *Anopheles*, even if the rises did not occur too soon after the cessation of the rain to allow of the necessary passage of the malarial parasite through newly matured insects. On July 29th the pools were again full of water, but only 1 out of 13 contained the larvae.

In August and September no larvae were found in the tanks, but a certain number were found in some of the pools, and the roadside drains also usually contained them, especially those in which grass protected them from the strength of the currents of water. During the latter month unprecedentedly heavy rain fell, 14 inches being registered on the 20th, and 10 more on the 21st, while over 40 inches fell in 7 days. Calcutta was flooded, and the area under examination suffered severely, being nearly completely submerged. It was not until the 27th of the month that I could get to the place, when I failed for the only time during the whole year to find a single *Anopheles* larva. Although all the pools were full of water, they must have been thoroughly scoured out by the torrents.

During October weekly examinations of the area were made so as to be able to closely watch the effect of the flood on both the larvae and the fever rate. The latter reached its maximum during this month, 260 cases being treated, or nearly three times as many as in the hot weather months; but this is usual at the break of the rains. On the other hand the *Anopheles* larvae were at their minimum, with the exception perhaps of the cold weather months, for while the tanks still remained free, most of the pools dried up before any larvae reappeared in them, so that only from 3 to 5 small pools were found containing larvae during this month, the total being smaller than in the few tanks which are infested during the cold weather months.

Further, they were only found in one-third of the drains during the first half of the month, so that the flood appears to have washed away all the larvae, while, on the cessation of the rains the pools and drains dried up before many fresh larvae had time to mature.

At the end of November only one *Anopheles* breeding-pool remained (which did not dry up until February) but the larvae began to appear once more in the tanks, although in much smaller numbers than during the hot weather, and they could be found therein throughout the cold weather months, and once more have begun to increase in them with the onset of warmer weather in March, so that there is no season of the year in this balmy climate when these noxious insects do not breed, although they are less prolific in the cold season than in the hot weather.

The numerous observations on the breeding places of *Anopheles* made by me in various parts of Lower Bengal in the course of tours at the end of the rainy season and during the cold weather and early hot weather months yielded precisely parallel results to those detailed above. They may, therefore, be taken as typical of Lower Bengal, which is the most extensive highly malarious tract in India.

The Importance of Tanks as Breeding-places for Anopheles.

The fact that tanks are the most important breeding ground of *Anopheles* in Lower Bengal must be emphasized, especially in view of Major Ross having recently stated that "it is well known that mosquitos do not breed in tanks, possibly because they are eaten by fish." Perhaps he did not examine them during the hot weather months in densely populated areas. My observations on this point have been fully confirmed by Dr Nield Cook, the Health Officer of Calcutta, and others. Further, most of the tanks in which I found them were swarming with fish, while I have already recorded an instance of *Anopheles* larvae being present in tiny shallow pools of a few square yards in extent in spite of small fish being also therein¹. The disappearance of the larvae from the tanks during the rainy season, their appearance in small numbers in them during the cold weather months, and their rapid increase in the hot season, are alike remarkable. The most probable explanation of these facts appears to me to be that

¹ Nuttall, Cobbett, and Strangeways-Pigg (*Journ. of Hygiene*, vol. i. p. 12) have made similar observations in England.

during the hot weather months the fish lie dormant at the bottom of the tanks, and the larvae can breed in safety, but during the rains they are more lively and rapidly destroy the insects. This is in accordance with what I saw on my rounds, for while no fish were seen to rise in the hot dry weather, in the rains on the contrary the water often appeared to be alive with them, while in the latter half of this season, when no larvae at all were found in the tanks, shoals of young fish were often seen swimming about close to the surface of the water, and these would have made short work of any larvae which might hatch out in the tanks at that season. However this may be, the fact remains that the maximum number of *Anopheles* mature in the tanks during the hot weather, and any plan for destroying these insects must take this point into consideration; and as far as regards Bengal, at any rate, it is not true that *Anopheles* only breed in small pools without fish.

*The Divergence between the seasonal prevalence of Anopheles
and that of Malaria.*

It will have been observed that the data given above relate only to the number of larvae found each month. Although the number of winged insects hatched out at any time will depend on the number of larvae, the number of adult *Anopheles* will also be affected by the favourableness or otherwise of the conditions which influence the length of existence in the winged state. Unfortunately it was impossible to search for mosquitos in the houses of the area under observation on account of the purdah system¹. My own residence is situated in the most healthy European quarter of Calcutta, *Anopheles* being seldom found there. However, at the same time that my searches for the larvae were made, Major Brown of the Indian Medical Service, who lives in a feverish suburb of Calcutta, made regular observations on the numbers of *Anopheles* in his house, and his results agree closely with my own in showing that while a few of the adult insects were met with in the cold weather months, yet they increased considerably in the hot weather months, when they attain their maximum, and decreased again on the onset of the rains, just as the larvae did, so that the two stages

¹ Under the purdah system the women of a household must not be seen by any European, and advantage is taken of this by natives to prevent the entry of Europeans into their houses for sanitary or even excise purposes unless due notice is given and other formalities complied with.

closely coincided, as indeed Celli also states is the case in Italy. As, further, in Italy the malarial fevers begin to increase in July, and attain their maximum in August, September and October, decreasing again in November and December, just as is the case in Lower Bengal, it might be expected that the prevalence of *Anopheles* would also coincide in the two countries. Turning again to Celli's recent work on malaria we find he states that in Italy new generations of larvae make their appearance in some waters in May, increase in June, and attain their maximum in the feverish months of July and August, and maintain it until the heavy autumn rains wash them out. If the tanks are left out of consideration, and only the pools and drains are considered, then the Italian conditions agree closely with those of Lower Bengal, and no doubt of other parts of India which are affected by the south-west monsoon⁽³⁾. This relationship is, however, completely altered by the marked increase in the number of *Anopheles* breeding in the tanks, this increase beginning in March and reaching its maximum in May at the season of minimal fever; decreasing in June, and practically ceasing in July, that is in just the months when malarial fevers begin to increase. I am inclined to think that the influence of the great heat of from March to May is the operative factor, which may act by preventing the majority of the insects which are hatched out in the hot season from surviving long enough to act as effective carriers of the malarial parasite. Certain it is that I have found it more difficult to keep *Anopheles* alive for more than a very few days in the hot weather than in the rainy season. Still the prevalence in the tanks must be taken into account in considering the feasibility of destroying these pests.

The rise of malarial fevers to a maximum in October, at the very time that the number of *Anopheles*' breeding places are decreasing, is probably accounted for by the steadily increasing number of infected adult insects in the houses throughout the fever season. These insects will continue to live as long as the temperature and other conditions are favourable. It is worthy of note that the rapid fall in the fever curve at the end of December occurred three weeks after the minimum temperature fell below 60° F., which is very nearly the lowest point it reaches in this genial climate, so that the diminished fever rate is probably due to the relative coldness being inimical to the local *Anopheles*. It is also remarkable that the rise of the fever curve in February was coincident with an unusual rise of temperature for the time of year, which again may have awakened the hibernating *Anopheles*

to a sense of their opportunities in justifying their name as being harmful. With the exception, then, of the remarkable and important prevalence of the *Anopheles* in the tanks during the hot weather, the other facts with regard to the seasonal distribution of these mosquitos and of malarial fevers respectively agree very fairly well, and are in accordance with recent observations in other parts of India⁽³⁾.

The Prophylaxis of Malaria in Lower Bengal.

We are now in a position to discuss the application of the various methods of preventing malaria which have been recently proposed on the basis of the mosquito theory. The fact that the highest authorities differ very widely in their advocacy of these measures indicates that, while none of them are perfect in practice, each may have its value in different conditions, and they may be most conveniently dealt with under separate headings.

1. *The destruction of Anopheles.* Very few authorities now consider it feasible to reduce malaria materially by the destruction of *Anopheles*, but as Major Ross is still of the opinion that in the end this will be the cheapest and most effective method⁽²⁾, it must be considered in the case of towns. The very frequent infection of even large tanks (and those I found infested by *Anopheles* varied between 10,000 and 200,000 square feet in surface area) would require a very large amount of oil or other material to disinfect them once a week throughout the hot weather, as well as a large staff, while the area examined is about one two hundred and fiftieth part of Calcutta, so that the difficulty of dealing with the thousands of tanks within the town and its suburbs would be very great. Unfortunately I found from inquiry that the owners of many of the tanks would not hear of any disinfectants being added to them for fear of harming the fish, so that the task of thus destroying the mosquitos in the tanks is an impossible one. The number of pools during the rains is also enormous, but much might be done in filling them up. More important and difficult to deal with are the open drains which line each side of every road. In the main streets, where they are lined with stone or brick, the current is sufficiently strong to wash away the larvae, but it is quite otherwise with the numberless earth-lined ones in the suburbs, which I found to form the most important breeding place for *Anopheles* during the rainy season, as well as near water stand-pipes in the drier times of the year. Some attempts to disinfect these by weekly applications of tar were made by Dr Cook, but in one instance

which I watched I found that a temporary decrease in the number of the larvae in the treated part, as compared with another part of the drain not so treated, was followed at the end of a week by the reversed condition of more *Anopheles* in the tarred portion, apparently due to numerous small frogs having been driven out by the tar, most of which had been subsequently washed away by the rain. The scores of miles of these drains in such a town as Calcutta will be at least as formidable to deal with as the tanks. It must also be remembered that the experiments of the Committee of the Royal Society⁽⁴⁾ have shown that artificial pools become infested with *Anopheles* within a few days, even when there are no breeding places in the neighbourhood, so that it would be of little use to try and destroy the larvae in a limited area of the town, as the breeding places would be very quickly reinfested from the surrounding parts. I regret, then, to have to come to the conclusion that it is not feasible to reduce very materially the number of *Anopheles* in Bengal towns by any practical method of destroying the larvae. Something in this direction can and should be done in the more favourably situated European quarters of our towns, but to attempt at a heavy cost to reduce the malaria of whole towns in this way in Lower Bengal is, I feel sure, certain to end in failure, which would do much harm by creating a prejudice against all measures based on the mosquito theory. Although I would deprecate the expenditure of the large sums that would be necessary to disinfect regularly the enormous and extensive mosquito breeding places in the tanks and drains of Bengal towns as being unlikely to meet with sufficient success to warrant the expenditure¹, yet this is no excuse for not doing as much as possible under the circumstances to lessen the number of breeding pools. The most important measure is to make use of the powers conferred by the municipal Act to make owners fill up small depressions such as will form suitable breeding pools during the rainy season. This I have always enjoined in my municipal inspections, while circulars have been issued in India calling attention to the importance of this simple proceeding, by which some good can be effected. This and any other such measures should be begun before the rains set in. It should also be clearly understood that the above remarks only apply to Lower Bengal, where the conditions are exceptionally unfavourable, while possibly much more might be successfully accomplished in drier

¹ It must be borne in mind that the total income of a Bengal town of 30,000 inhabitants for all sanitary and municipal wants is but some £2,000, so that the necessary expenditure can only be met at the cost of omitting other essential sanitary measures.

provinces, although these will be less malarious than Bengal and Assam. There is one reform which might and ought to be carried out in India, and that is the prohibition of rice-fields within the limits of towns, or within a mile of their boundaries. This measure has been found of value in both Italy and Spain. *Anopheles* have been found breeding in rice-fields in Madras.

2. *The Use of Quinine.* The suggestion of Koch, Grassi, and others to destroy the malarial parasite in the blood of the comparatively small number of persons who carry it during the season of minimal fever prevalence, is worthy of careful trial in places where there is a very marked and prolonged season of absence of new infections, and where the inhabitants are sufficiently intelligent and well to do to understand and carry out the method, and efficient medical supervision is available. The wholesale experiments in Italy will be watched with the greatest interest. Unfortunately none of these necessary conditions are met with in Bengal, where, even during the months of minimal fever incidence, the number of fever cases, mostly no doubt relapses, still form about one-fourth of the numbers at the maximal season, so that the complete use of this method is impracticable in this country. The sale of pice¹ packets of quinine through the post-offices has done much to bring this drug within the reach of the rural inhabitants, but unfortunately they are too poor to use it regularly as a prophylactic, even if they could be persuaded to do so.

On the other hand, in the case of Europeans, I have for some years been strongly in favour of the prophylactic use of this drug during the malarial season or on visiting malarial places, and believe this practice was the main cause of my escaping malarial fever during a year's work in the most unhealthy districts of Assam, investigating the epidemic malarial fever of that province, locally known as kala-azar (black fever), although I never used mosquito curtains on account of the heat. I prefer ten-grain doses twice a week, or for those who are specially susceptible to the drug, five grains every other day.

It is from this point of view, too, that studies of the effects of meteorological conditions on the curve of the incidence of fever are of great value, for on account of the frequency of recurrences of malarial fever secondary attacks must be more common than primary infections, and any data by which the likelihood of a recurrence can be foretold will be an important guide to the successful prophylactic

¹ A pice is equivalent to a farthing of English money.

use of quinine. I have elsewhere pointed out that in places with a low ground-water level during the dry seasons, and where it rises rapidly and fluctuates considerably during the rains, the fever curve rises with each rise of the ground-water and falls with each decline⁽⁵⁾. Again, in Lower Bengal, as well as in other places, such as Lucknow in the North-West Provinces, where there were no such violent fluctuations of the ground-water, the fever curve rises during the breaks in the rains, or under just the opposite conditions to the former case. Such variations of the fever curve must largely depend on causes which predispose to a relapse in persons already infected by the malarial parasite, and a knowledge of these facts will allow of quinine being taken as a prophylactic at the time of heavy rain in the former case, and during breaks in the rains under the latter conditions: a plan which I have repeatedly acted on with, I believe, beneficial results, and whose value is not affected by its discovery being independent of and prior to the establishment of the mosquito theory. The precise explanation of the facts noted is not quite so clear, but breaks in the rains are always most trying on account of the great damp heat, which by lowering the resisting power might easily predispose to a relapse, while in the higher parts of Chotta Nagpur, where I met with the most marked instance of the rise of fever with heavy rain, this was accompanied by a very great and rapid fall in the temperature, sometimes amounting to 30° F. in a few hours, such as might easily cause a recurrence of fever which a timely dose of quinine would prevent or greatly mitigate.

3. *Protection from mosquito bites.* Not much need be said under this heading, as everyone will admit the advisability of using mosquito curtains in malarious places, although it is not practicable in ordinary civilised life to stay within them from before sunset to after sunrise. The protection of houses by wire netting is beyond the means of the poor, and will not be advisable in the case of Europeans except in a few very malarious spots, on account of the great obstruction caused by netting to the free circulation of breezes through the house, which is such an important factor in keeping them from getting stiflingly hot in the tropics, and so rendering life bearable. The application of substances to exposed parts of the body to prevent the insects biting may also be of use, and I have known them successfully applied to keep off swarms of mosquitos when men are sitting up at night over a kill for tiger. Insecticide powders are also being burned with advantage in houses in India for lessening the number of mosquitos.

4. *Drainage operations.* Extensive drainage operations have fre-

quently been carried out in India for reducing malaria, a successful example of which will be found in a paper by Major Dyson, I.M.S., in the Transactions of the first Indian Medical Congress, 1895, p. 283. It is equally important to prevent places being made more malarious by raising the ground-water level through obstruction to surface drainage in the construction of embankments for railways and canals, without allowing sufficient waterway through or under them.

It is, however, only under exceptional circumstances that large drainage operations can be undertaken in India for the lessening of malaria, and Lower Bengal is particularly unfavourably situated for such works on account of the very peculiar circumstance that the water in most of the rivers rises to a greater height than the surrounding country, which must be flooded by them more or less during the height of the rainy season, sufficient drainage being impossible.

But there is another aspect of the question which demands more attention, and that is the condition of the roadside drains in towns and large villages. I have already pointed out earlier in this paper that the unlined roadside drains form the most important breeding place for *Anopheles* in the rainy fever season, while they are most difficult to disinfect. On the other hand, I have never been able to find the larvae in brick or stone-lined drains with a good current of water flowing through them. It is obvious, then, that the lining of the roadside drains in small towns will be an important measure in lessening the number of *Anopheles* breeding in close proximity to the most thickly distributed houses of the place. I have also observed that the larvae are most easily found in such unlined drains as have grass growing in them, especially after heavy rain, the grass evidently affording the larvae considerable protection from the force of the current. The cleaning out of all such drains as cannot be lined at regular intervals during the rainy season will, then, also be an important sanitary measure from this point of view, and these suggestions should be carefully acted on by municipal authorities in Bengal as well as in other places in which such drains are found to harbour the larvae of *Anopheles*.

5. *Segregation measures.* The discovery by Koch in New Guinea, and by Stephens and Christophers in West Africa, of the frequency with which infants harbour the malarial parasite in their blood with little or no symptoms, strongly supports their conclusions that native children are an important source of infection to Europeans living in

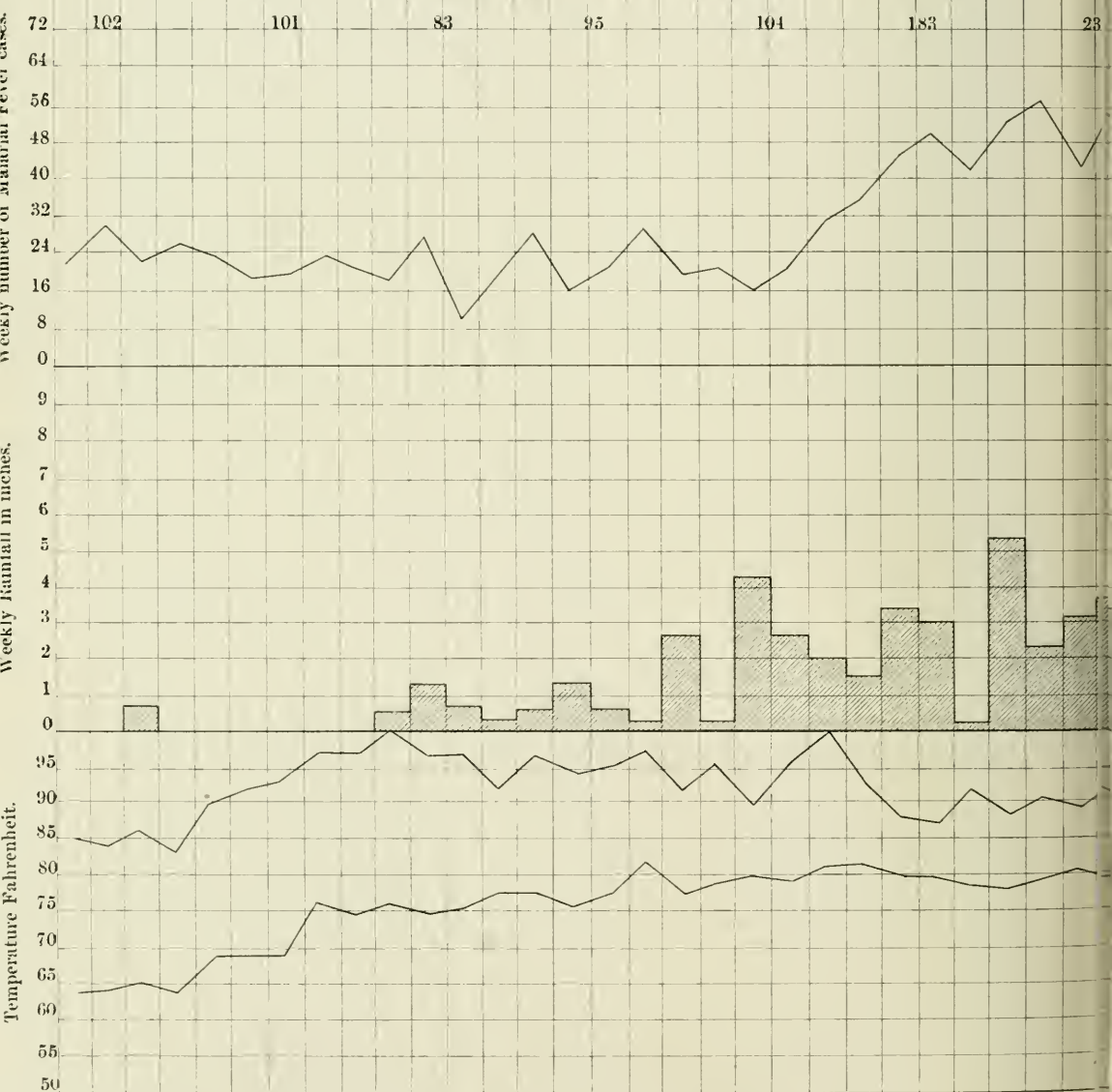
their neighbourhood, and indicates the necessity of the European quarter being at some distance from native huts. This, together with the selection of sites for rest-houses at some distance from the nearest village, instead of close to it, should be carried out in India. During a recent tour in a remote part of the Tributary States of Orissa I came across a sub-divisional station which was so malarious that a new site eight miles off was being laid out to which the offices, etc. were to be shifted from the unhealthy spot. I was just in time to get the authorities to make some slight alterations in their plans so as to ensure the native quarter being kept at a distance from the Europeans' houses, and the result will be watched with interest.

There is one other set of circumstances in which the principle of segregation may be of the utmost value, and one too which I was able to recommend and carry out with very great success in 1897 before the discovery by Major Ross of the communicability of the disease through the mosquito, and which I may be pardoned for referring to here. I refer to the epidemic malarial fever of Assam, or kala-azar⁽⁶⁾, which I declared as early as 1897 to be a very intense form of malarial fever, slowly and probably indirectly communicable from one person to another in some way which was not quite clear, but which I did not think at the time was transmitted by means of the mosquito, because I felt sure that the vehicle of infection was not water, as was suggested in Manson's original hypothesis. I gave numerous instances of the disease being introduced into a village by a person infected with this particular type of malarial fever, and in which the first people to get it were those living in the same house as the imported case. Ross a year or two later confirmed my views after a short personal investigation of the disease in Assam, and generously gave me full credit in his report for my "boldness in declaring the communicability of paludism," while his brilliant researches have settled the difficulty as to the exact way in which the disease is spread; and now that malaria is known to be actually inoculated by the mosquito, I fully accept this method of infection as explaining the facts with regard to the spread of this peculiarly intense form of malaria in Assam. I have elsewhere described⁽⁷⁾ the success of the measures which I advocated for the control of the disease, and have given instances in which by moving out all those who had fever, together with their households, during the cold weather when the disease was at its minimum, several coolie lines remained quite or very nearly free from this very fatal form of malarial fever throughout the following fever seasons. In another instance where

420^m

1900.

	February.	March.	April.	May.	June.	July.	Aug.
<i>anks</i> {	some infested	half infested	one-third infested	two-thirds infested several increasing	one-sixth infested with few only	only two infested	ni
<i>ools</i> {	one infested rest dry	three infested rest dry	all dry	one infested rest dry	4 out of 7 infested	10 out of 14 infested	9 out of 14 infested
<i>ains</i> {	all dry	dry	dry	dry	two infested	all infested	all infested
<i>eral</i> {	scanty	numerous	numerous	maximum	much fewer but more diffused	more widely distributed	some scattered

Chart of Monthly prevalence and distribution of *Anopheles* larvæ, and Weekly

1901.

September.

October.

November.

December.

January.

February.

March.

nil.

nil.

one sixth
infested

few infested

few infested

few infested

increasing } Tanks

nil after flood
6 out of 11
infested5 out of 7
infested
rest drytwo infested
rest dry

one infested

one infested
rest dry

all dry

all dry } Pools.

half infested

one-third
infested in
first half only

all dry

all dry

all dry

all dry

all dry } Drains

somewhat
scanty

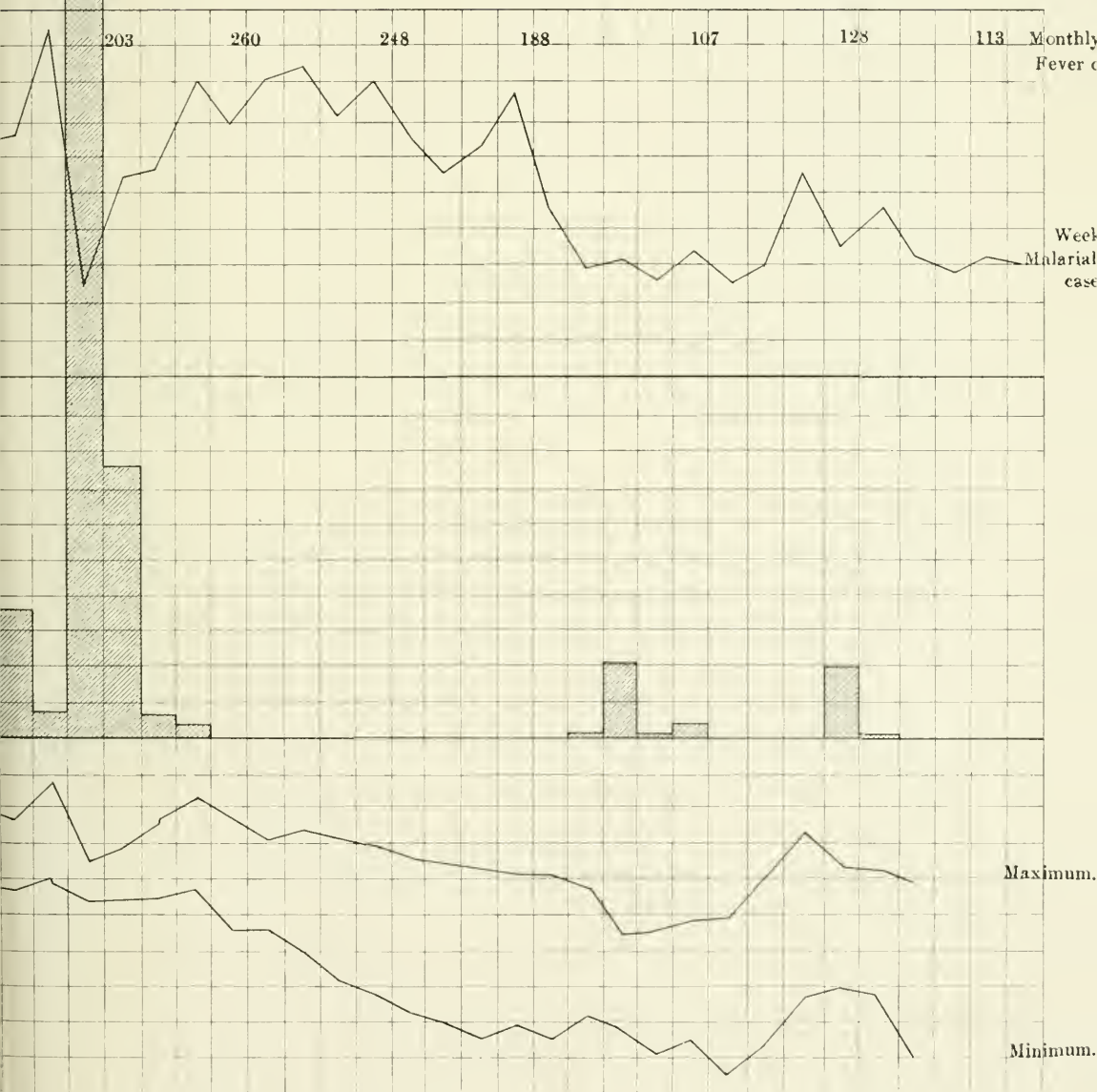
scanty

scanty

scanty

scanty

scanty

increasing } General
Prevalence

more than half the households were found to have the infection the remaining healthy ones were removed, and placed, together with a large number of freshly imported coolies, in a new line some three-quarters of a mile from the old one, with equally satisfactory results, none of them becoming infected during the next four years. These measures have in fact been so successful that recently I was unable to get any post mortem material of kala-azar cases from the very gardens which a few years back were having over a hundred deaths a year from this disease. I may, then, fairly claim to have recognised the infectious nature of this virulent form of malarial fever. I moreover successfully carried out measures of segregation two years before the brilliant work of Ross afforded a solid basis for the mosquito theory, and showed that the infection was conveyed through the air, as I thought, and not through water, the mode of entrance being, however, inoculation by mosquitos, and not inhalation through the lungs, which I suggested as the most likely hypothesis at that time. The value of segregation, under special circumstances at any rate, is, then, proved, and when it is applicable it is a most important measure in preventing the spread of malaria, especially in its very fatal epidemic forms, such as the Mauritius epidemic in 1866—67, the Burdwan fever, or as I prefer to call it the epidemic malarial fever of Lower Bengal, of the fifties, sixties, and seventies, of which I have shown elsewhere⁽⁸⁾ there is some reason to believe the Mauritius epidemic may have been an offshoot, and the present epidemic malarial fever of Assam, now happily on the wane.

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THE ELKTON MILK EPIDEMIC OF TYPHOID FEVER.

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ELKTON, the county seat of Cecil county, Maryland, is a town of 2542 inhabitants, built upon a red clay soil, rather flat as to surface, but sloping gently toward the Elk river. This small stream is not navigable, is but slightly influenced by the tides, and is only about 25 feet below the highest part of the town.

The public water supply is obtained from the river, a mile and a half above the town. The stream above the waterworks flows through a farming country, and is unprotected. There is no impounding reservoir, the water being distributed by the pressure from a stand-pipe 100 feet high. The *Bacillus coli* has been found on several occasions in the town supply, but not during the year 1900. The quality of the water is at no time above suspicion.

The town is unsewered. Surface-closets are largely used, and the better houses are supplied with water-closets discharging into cesspools, some of them uncemented, and some probably penetrating the 15 feet of clay into the gravelly sub-soil.

The majority of families obtain their drinking water from private wells 20 to 30 feet deep. Typhoid fever has been present in Elkton every year, and two epidemics have occurred, one in 1884, which was traced to a dairy, and the one in 1900 which is now under consideration.

Of the four dairymen delivering milk in Elkton in 1900 the largest business was probably that of A., whose farm is about three miles distant.

On his way to town every morning A. obtained milk from two other farms, both of which remained free from sickness in 1900. Whether the milk was distributed mixed or unmixed could not be learned.

More than half of A.'s supply was obtained from his own herd of 22 or 23 cows. A.'s manner of handling milk was not better nor worse than is usual with small dairy farmers. The well is about 15 feet from the barn-yard fence, and beside it stands a large wooden trough in which the cans were set to cool the fresh milk. This water served all the purposes of the family and the dairy. Two samples of water were obtained from this well at different times in October, 1900, and two determinations were made upon each sample. *B. coli* was isolated in all four instances.

In September, 1900, a case of typhoid fever occurred on the adjoining farm B. Mrs A., wife of the dairyman, assisted in nursing the case at B. during the two or three weeks preceding death, which occurred on Oct. 5th. Before this date Mrs A. and her son, aged 15, were ailing, but the boy continued to milk the cows, and Mrs A. to prepare the milk for market, until about Oct. 8th, when both were obliged to cease work. A homœopathic physician was called and pronounced a diagnosis of "Summer grip." Neither of them was severely ill, though both when seen by myself on Oct. 28th were pale, weak and emaciated. The older son, aged 21, suffered a more severe attack early in November, the same diagnosis being made. The father alone of the family remained well. They refused to submit blood specimens for the Widal test, and the attending physician, though admitting a doubt as to the nature of the illness and professing his belief in this means of diagnosis, withheld his consent to a blood examination.

The notification law is fairly well observed in Elkton, and previous to this time there had been but three cases of typhoid fever, all in one family, using the public water-supply. These cases, A. D., age 32, August 12th; E. D., age 71, Sept. 12th; and M. D., age 36, Sept. 19th, were all in the care of the Health Officer, Dr Bratton, who attributed the first case to an infected well at a house which the young man frequented. The two later cases cannot be so accounted for. Possibly they belong to the milk outbreak, though it is very unlikely. The family began to be supplied with A.'s milk late in August, at which time A.'s milk was probably not infected. The excreta in all the D. cases were disinfected.

On October 11th three cases of typhoid fever were recognized in Elkton; on the 12th, one case; 13th, two cases; 14th, three cases; 15th, three cases; 16th, three cases; 18th, six cases. These cases were attributed by the local Health Officer, Dr Bratton, to A.'s milk. The evidence on the 18th was as follows: Infected households, 17; using

the public water-supply, 11; using private wells, 6; using A.'s milk, 17. Among the 21 cases there was but one in which doubt was felt about the use of A.'s milk. Mrs M., aged 63, attacked on Oct. 12th, was not a regular customer of any milkman. There were but two persons in her house, neither of them caring for milk, but they did occasionally stop A.'s waggon and buy cream and milk for table use (in coffee and on fruit). A date when milk had been bought within two or three weeks preceding this illness could not be fixed.

The Health Officer considered this evidence strong enough to warrant advising A. to discontinue the sale of milk pending further investigation. A. continued his business, though his customers steadily forsook him. He ceased selling on Oct. 28th. On that date the figures were as follows:

Infected families, 32; using the public water-supply, 18; using private wells, 14; using A.'s milk, 32. Total number of cases, 39.

Among these was the case of Mary D., aged 10, who was ill when brought into Elkton on Oct. 20th, from a farm where her mother was employed as a domestic. Her home in Elkton was supplied with A.'s milk, and had a private well. Her sister, Jennie D., who was not out of town, became ill on Oct. 24th. It was subsequently learned that A. left milk daily for Mary D.'s use at the farm. This farm-house, infected by A.'s milk, is not included in our figures.

The outbreak subsided after Oct. 29th, the record being (Jan. 1st, 1901) as follows:

Infected houses, 39; using town water, 21; using private wells, 18; using A.'s milk, 39. Total number of cases, 64.

In the S. household there were three cases, the last of which occurred 35 days after the last delivery of A.'s milk. This third case is not included in our figures. The S. family, besides two undoubted milk cases, adds to the record the interesting case of Alice M., who visited the town for two days only, stopping with the S. family on Oct. 5th and 6th. She returned on Oct. 7th to her home in New Jersey, where she failed with enteric fever on Oct. 14th. The first case in the S. family fell ill on Oct. 16th.

But one case was traced to mixed milk. Mrs B. was supplied by a neighbour who sold milk in a very small way, part of her stock being regularly obtained from A.'s waggon.

In the W. family was a negro servant, whose chief food consisted of oatmeal and milk. She left Elkton about the middle of October, and

went to Glasgow, Delaware, where she became ill, and died of typhoid fever on Nov. 13th.

In the B. family was a married daughter, aged 38, who left Elkton late in October to visit friends in Pennsylvania. About 10 days after leaving Elkton she was attacked with typhoid fever.

The most interesting series of cases, and one which supports in a striking manner the causative relation of A.'s milk to the epidemic, occurred at the jail. The jailer's wife, aged 35, and her two sons, aged 17 and 13, had typhoid fever. None of them admitted the use of A.'s milk as a drink; two of them used it in coffee and on oatmeal and raw fruit, and one of them only as ice-cream. Ice-cream was made regularly once a week from A.'s milk. No milk was given in any form to the prisoners. The iron bars kept the prisoners in, and the milk out. The inmates of the prison proper numbered from 15 to 20 during the period of this outbreak. There were, however, two negro men among them who were not criminals. One was an epileptic and the other an insane man. These two slept in the jail, and during the day were employed in domestic service and in errands about the town. The jailer, anxious to have his insanitary building condemned by the authorities, declared that these two men had all their meals with the prisoners, but it is certain that they had daily access to the family provisions, and it is most significant that these two men were both attacked with typhoid fever in the first week of November. Here were some 24 persons exposed under one roof to conditions identical in all respects, except as to a single article of diet and as to the visits in the town. Down to Oct. 29th, A.'s milk reached as far as the prison bars which divided the inmates into two classes; six on this side, who could get milk, and of whom five had typhoid fever; and eighteen on the other side, who could not get milk, all of whom escaped. No other case appeared at the jail until about the 8th of December, when a white man, after ten days' incarceration, developed typhoid fever. He was a tramp, had been in the town but a few days before his arrest, and his history threw no light upon the source of his infection. This case was followed by two others which could not be traced to any source outside the jail.

Summing up, we find first that the town of Elkton had a single infected house on Oct. 1st. On Oct. 5th, a death from typhoid fever occurred on a farm 3 miles from town. On the adjoining dairy farm, two persons, known to have been in intimate contact with the preceding fatal case throughout its course, became ill with a fever during the first week of October.

Beginning on Oct. 11th, the town of Elkton suffered an outbreak of typhoid fever numbering 39 cases in the first eighteen days, during which time the dairyman's business continued. Twenty additional cases occurred within the three weeks following the milkman's last sale, and four cases from the latter date to the end of the epidemic. There were in all 39 houses infected, all supplied with A.'s milk. In the 39 houses occurred all the typhoid fever which appeared in Elkton during the last three months of 1900. Taking into account the three cases which developed shortly after leaving Elkton, the one case which came to Elkton while ill, and the three cases in jail in December, we have 64 cases of typhoid fever, of whom 61 were consumers of A.'s milk, and the 60th case fell ill within 18 days of A.'s last delivery. After a further interval of 13 days came the first of the four cases which are not attributable to A.'s milk. None of these happened within the period of immediate influence of A.'s milk, namely, from the appearance of fever at A.'s farm, in the first week of October, down to Nov. 21st, twenty-three days after the milk ceased to be sold.

In the outbreak two deaths occurred, both women; one white, aged 56, and the other a young coloured woman.

Three patients who recovered had suffered previous attacks, one in the milk epidemic of 1884, one in 1893, and one in 1898. The last named was a prisoner and probably not a milk case.

Period of Incubation.

The case of Alice M. shows a period of incubation not longer than 9 days; Oct. 5th to 14th. Cases in which the date of infection can be accurately determined are somewhat rare.

Emil Janchen¹ (1898) reported an outbreak in which the date of infection was known, and the symptoms of onset were marked. A number of regiments returning from autumn manoeuvres passed, without halting, through a village where typhoid fever was prevalent. These troops showed no special incidence of typhoid fever. One regiment, however, halted in this village, on a hot day, after a tiresome march, and the soldiers drank freely of the infected water. This occurred on the 10th of September, and the symptoms of invasion of typhoid fever appeared on succeeding dates as follows:

¹ *Wiener klin. Wochenschr.* Jahrg. xi. p. 667.

Sept. 12th	2 days later	3 cases
" 13th	3 " "	7 "
" 14th	4 " "	6 "
" 15th	5 " "	4 "
" 16th	6 " "	4 "
" 17th	7 " "	5 "
" 19th	9 " "	1 "
" 20th	10 " "	2 "
" 21st	11 " "	1 "
" 22nd	12 " "	1 "
" 23rd	13 " "	1 "
" 24th	14 " "	1 "

These 36 cases completed the outbreak. The numerical strength of the regiment is not given, but it may be assumed to have contained 1200 men (the peace footing in Germany) of susceptible ages, giving an attack rate of 3 per cent. Fatigue probably shortened the period of incubation in these cases.

Stokes and I¹ reported in 1898 an outbreak in which the date of infection was precisely determined. In a suburban community of 400 persons, the pump, which supplied water of good quality, became disabled on the night of July 3rd. The supply for July 4th was drawn from a well which contained *B. coli*. On July 5th the regular supply was restored. The use of infected water was, therefore, confined to the 4th of July. On the 9th, 10th, and 11th of July some 25 persons were seized with gastro-intestinal symptoms. All had diarrhœa, and nearly all had fever. One had fever, bloody stools, and loss of weight; two had delirium, chills, fever and diarrhœa. All recovered in from 3 to 7 days. None of these were regarded as cases of typhoid fever. On July 27th two young ladies, and on August 1st a third, fell ill with typhoid fever. Other sources of infection than the water supply of July 4th were carefully excluded. Here, after one day's exposure, we have incubation periods of 23 and 28 days. The attack rate was 75 per cent. among 400 people of all ages.

In the Marylebone epidemic² of 1873 is recorded the case of a child who drank the infected milk but once. On the afternoon of July 19th she is said to have drunk two pints, and her illness began on July 24th; a young subject, massive dose, and short incubation.

¹ *Report of the State Board of Health of Maryland*, 1898, p. 103.

² *Report of the Medical Officer to the Local Government Board*, 1874, No. II. pp. 103—136.

The Clifton epidemic¹ of 1897 included the case of a child of 9, who drank the infected milk on but one day, her attack following "at the end of a week."

In the Mont Clair, N.J., outbreak (1894)², 9 persons were said to have drunk the infected milk once only. These fell ill from 14 to 27 days later. In this epidemic the fatality was 13 per cent., and 28 out of 44 families using the milk were attacked.

The Great Harwood outbreak³ reported by Sargeant included one person whose attack came two days after a single glass of the infected milk. In contrast with this was the case observed by Power⁴, in which the attack followed a single glass of milk after an interval of 3 weeks.

In the Elkton epidemic the first 21 cases seem to indicate periods of incubation averaging under rather than over 14 days. In the 60th case the time from the last drink of milk to the first visit of the physician was 19 days. If this case be regarded as the last one due to milk, it probably indicates for this outbreak the longest period of incubation.

Attack Rate.

The dairyman claimed to be regularly supplying 80 houses. This statement was made at a time when his interest would have been served by proof that a great part of his route was free from typhoid fever. On this basis the house incidence was 48.75 per cent. In the 39 infected houses were 180 people, so that the attack rate in these houses was $33\frac{1}{3}$ per cent. The case rate was 1.54 per house. Taking the milkman's estimate of 80 exposed houses, with 4.6 persons per house, we should have 368 persons exposed, giving a general attack rate of 16.3 per cent. The light fatality, 3.3 per cent., suggests a contagium of slight virulence; but milk as a vehicle usually means large dosage, and the dose is related to the period of incubation, the attack rate, and perhaps to the fatality.

In the Clifton epidemic, before referred to, it was observed that the attack rate was highest among the people who received the unmixed

¹ D. S. Davies, *Lancet*, 1897, vol. II. p. 1442.

² R. C. Newton, *New York Med. Record*, vol. XLV. p. 713.

³ *Lancet*, 1895, vol. I. p. 1328.

⁴ Quoted by Dawson Williams from Report, 1892, of a Committee of the Clinical Society of London to investigate Periods of Incubation etc., *Twentieth Century Practice*, vol. XIII. p. 371.

milk of the infected dairy. Two other milk-vendors received a small part of their supply from the infected milk. The houses served by these two vendors with mixed milk were attacked at the rate of 41·8 per cent. and 38·5 per cent. respectively, as against 54·4 per cent. for the unmixed milk.

In an epidemic recently reported at the Iowa State College¹, the attack rate was 8·8 per cent., the fatality 4·8 per cent., and the striking observation is made by Dr Kennedy that among the football players, who were served a double allowance of milk, the attack rate was 50 per cent. The whole number of cases, 42, appeared in a period of 27 days, the last two cases being separated from the last service of infected milk by a period of 19 days.

The influence of dosage is also apparent in the incidence of the disease upon children. Cameron² reported an outbreak, at a barrack, due to milk, in which the attack rates were as follows :

Among 600 constabulary,	20 cases,	3·3 per cent.
„ 40 women,	2 „	5 „
„ 100 children,	10 „	10 „

The fatality was 15·6 per cent., all falling on the men. In the milk epidemic at Stamford, Connecticut³, 34·8 per cent. were between 1 and 10 years old, and 16·8 per cent. were 5 years old and under. In the Waterbury outbreak⁴, 23·3 per cent. of cases were 10 years old and under. In our epidemic at Elkton there were 19 cases between the ages of 20 months and 10 years ; 31·66 per cent.

I have to acknowledge particular indebtedness to Dr Howard Bratton, Health Officer of Cecil county, for important details concerning this interesting epidemic.

¹ *Iowa Health Bulletin*, vol. xiv. p. 91.

² *Dublin Journ. of Med. Sci.* Nov. 1899, vol. cviii. p. 330.

³ H. E. Smith, *Report of State Board of Health of Connecticut*, 1895, p. 168.

⁴ H. E. Smith, *Ibid.* 1890, p. 248.

THE NEUTRAL-RED REACTION AS A MEANS OF DETECTING BACILLUS COLI IN WATER SUPPLIES¹.

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THE object of the experiments detailed below, which were made at the suggestion of Dr W. Hunter, was to ascertain whether media containing neutral-red afford a rapid means of detecting *Bacillus coli* in water and estimating the number present.

In 1898 Rothberger² discovered that *B. coli* reduces solutions of neutral-red, the colour changing to canary-yellow, accompanied by green fluorescence. Since *B. typhosus* does not do so a valuable means of distinguishing between these two organisms was afforded. Scheffler³ has confirmed Rothberger's work, extending the investigation to a large number of races of *B. coli*, obtained from different sources. With all these the reaction was constantly obtained, and he considers it so characteristic that any organism which fails to give it may be excluded from the *coli* group. Hunter⁴ has recently obtained identical results, and has further shown that *B. enteritidis* (Gaertner) also reduces neutral-red—a fact which places this bacillus closer to the *coli* group, and separates it more sharply from *B. typhosus*.

Rothberger⁵ found that certain anaerobic bacteria—*B. tetani*, *B. anthracis symptomatici*, and *B. oedematis maligni*—have the same power of reducing neutral-red, and Scheffler mentions that he separated from water and from faeces several species of micro-organisms which produce a green fluorescence, the part of the reaction on which he appears to lay most stress. Unfortunately he gives no hint as to their nature, beyond stating that they were not *B. coli*. On the other hand Rothberger and

¹ MS. received April, 1901.

² *Centrabl. f. Bakteriöl.*, vol. xxiv. p. 513, 1893.

³ *Ibid.*, vol. xxviii. p. 199, 1900.

⁴ *Lancet*, March 2, 1901, p. 613.

⁵ *Centrabl. f. Bakteriöl.*, vol. xxv. p. 69, 1899.

Hunter have tested most of the aerobic pathogenic organisms with negative results. Thus the *Staphylococcus*, *Streptococcus*, *Pneumococcus*, *Friedländer's Bacillus*, *B. diphtheriae*, *B. pyocyaneus*, *B. mallei*, *B. anthracis*, *Vibrio cholerae*, and many of the allied *Vibrios* failed to alter neutral-red.

I found that *B. tetani* and *B. oedematis maligni* produced in glucose-agar the same appearances as *B. coli*, even when the surface of the medium was exposed to air. With all three a layer of unreduced red was left at the top of the tube. In bouillon, however, the anaerobic bacilli only produced the reaction when oxygen was excluded. A reaction due to these organisms would thus appear to be easily distinguished, although we must remember that anaerobic bacteria have been observed to develop in cultures exposed to the air when associated with aerobic bacteria.

One organism isolated from tap-water—apparently a variety of *B. mesentericus*—was observed to change the red to a dull orange colour, both in bouillon and glucose-agar. The change began on the second day of incubation. The surface layer was, however, the part first affected, while with *B. coli* the change begins at the bottom of the tube, and in glucose-agar never reaches the surface. In a tube inoculated with both organisms it is possible to distinguish the two reactions in the upper and lower layers, when the bright yellow due to the *coli* contrasts with the dull orange of the *mesentericus*. The latter also acts much more slowly, and does not form gas in glucose-agar.

A number of other water organisms were tested, none of which gave the reaction. My experiments, so far as they have gone, seem to indicate that a water producing a typical canary-yellow colour in neutral-red media, within 48 hours in bouillon, and accompanied in glucose-agar by green fluorescence and gas-formation, may be considered to contain *B. coli*. At any rate in every case in which these appearances were obtained further examination revealed the presence of an organism with all the essential characters of the *coli* group.

A large number of experiments were made to test the delicacy of the reaction as an indication of the presence of *B. coli*. Flasks containing a known amount of sterilised tap-water were inoculated with varying quantities of 24-hour old bouillon cultures (themselves inoculated from old agar cultures) of *B. coli*. From each of these flasks quantities of 1 c.c. were added to tubes of bouillon and glucose-agar containing 1% of a saturated watery solution of neutral-red. The tubes were afterwards incubated at 37°. At the same time agar plates

TABLE I.

	FLASK A. Dil. $\frac{1}{1000}$	FLASK B. Dil. $\frac{1}{10,000}$	FLASK C. Dil. $\frac{1}{100,000}$	FLASK D. Dil. $\frac{1}{1,000,000}$	FLASK E. Dil. $\frac{1}{10,000,000}$	FLASK F. Dil. $\frac{1}{100,000,000}$	FLASK G. Dil. $\frac{1}{1,000,000,000}$	FLASK H. Dil. $\frac{1}{10,000,000,000}$
	1000 c.c. of sterile tap-water+1 c.c. bouillon culture <i>B. coli</i>	1000 c.c. of sterile tap-water+1 c.c. coli bouillon	1000 c.c. of sterile tap-water+40 c.c. of coli bouillon	1000 c.c. of sterile tap-water+1 c.c. of flask A	1000 c.c. of sterile tap-water+1 c.c. of flask B	1000 c.c. of sterile tap-water+1 c.c. of flask C	1000 c.c. of sterile tap-water+1 c.c. of flask D	1000 c.c. of sterile tap-water+1 c.c. of flask E
Neutral-red in 24 hours	1 c.c. of this dilution in each tube 1. + 2. + 3. +	1. + 2. + 3. +	1. + 2. + 3. +	1. + 2. + 3. +	1. + 2. + 3. +	1. + 2. + 3. +	1. + 2. + 3. +	1. + 2. + 3. +
Neutral-red glucose-agar in 24 hours	1 c.c. per tube 1. + 2. + 3. +	1. + 2. + 3. +	1. + 2. + 3. +	1. + 2. + 3. +	1. + 2. + 3. +	1. + 2. + 3. +	1. + 2. + 3. +	1. A few bubbles in 24 hours 2. Ditto 3. Ditto All three gave yellow fluid in 24 hours
No. of colonies on agar-plates	1 c.c. in each plate 1. } Innumerable 2. }	1. } Innumerable 2. }	1. } Innumerable 2. }	1. } Numerous 2. }	1. } 30-40 2. }	1. } 10-20 2. }	1. } 1-5 2. }	1. } 1-2 2. }

containing corresponding amounts of the water were prepared, and the colonies counted after 48 hours' incubation. Uniformity in method was thus obtained, and the results of many observations were fairly even.

It was evident that neutral-red affords a very delicate test. In bouillon a reaction could be constantly obtained within 24 hours even with dilutions corresponding to from 1 to 5 organisms per c.c. It consisted of a diffuse canary-yellow colour which in extreme dilutions did not reach the surface of the fluid in 24 hours, while the lower parts had a more orange tint than when *B. coli* was plentiful. If larger amounts of the culture were added complete change occurred within 12 hours. In glucose-agar the reaction was much less prompt, and with extreme dilutions it took 5 or 6 days for a complete reaction (medium broken up by gas-formation, and coloured yellow along with greenish fluorescence except at the upper $\frac{1}{2}$ inch) to develop. Gas-bubbles appeared first, usually within 24 hours, then a yellow fluid in the spaces at the bottom of the medium, and finally the full reaction.

It is unnecessary to detail the results of each series of dilutions. The above table, representing one series, will serve as an illustration.

In the table the sign + indicates a positive reaction irrespective of whether there was complete yellow coloration of the glucose-agar, or merely the appearance of yellow fluid at the bottom of the tube.

The experiments are summarised in Table II.

TABLE II.

Dilution	$\frac{1}{1000}$ to $\frac{1}{1,000,000}$	$\frac{1}{100,000,000}$	$\frac{1}{1,000,000,000}$	$\frac{1}{10,000,000,000}$	$\frac{1}{100,000,000,000}$
Neutral-red bouillon	Constant reaction in 24 hrs.	Constant reaction in 24 hrs.	Reaction generally in 24 hrs. Absent in about 10% of observations	Reaction in 24 hrs. in 40%. No reaction in 60%	Reaction in 24 hrs. very rarely
Neutral-red glucose-agar	Reaction in 24 hrs. complete yellowness or yellow fluid only	Reaction in 24 hrs. Medium broken up and yellow fluid in bottom of tube	Reaction in 24 hrs. in 25%; in 2-5 days in 50%. No reaction at all in 25%	Reaction in 48 hrs. in about 50%	No reaction
No. of colonies on agar plates	Very numerous	9-20	1-5	0-2	

It will be noticed in the tables that the colonies which developed on the agar plates are more numerous than corresponds to the increasing dilution. This was doubtless due to the rapid multiplication of the bacteria while the dilutions were being made.

These experiments were made with pure cultures of *B. coli*, and it was of course necessary, before applying the method to the examination of water-supplies, to determine how far the presence of other organisms might retard or alter the reaction. The time at my disposal only permitted a few experiments in this direction.

The whole series of flasks described in Table I. was prepared again, but this time *unsterile* tap-water was used—with the result that in neutral-red broth as far as Flask G (1 to 5 *coli* organisms per c.c.) all the tubes showed the reaction in 24 hours. Flask H, however, gave a reaction in only one out of three tubes, the others remaining unchanged in colour even after 48 hours. In glucose-agar an inhibitory action was more apparent, as Flask F (10 to 20 *coli* organisms per c.c.) gave a reaction in only one tube after 24 hours. The others were delayed five days, while those from Flasks G and H remained unchanged after six days. It should be added that, as the tap-water itself sometimes gave a reaction when 1 c.c. was added to a tube, the dilutions in the flasks were made double the strength given in Table I., so that it was only necessary to use .5 c.c. for inoculating each tube. This amount of pure tap-water rarely gave a reaction.

In another experiment a large carboy of 40 litres capacity was filled with unsterilised tap-water. To this was added .004 c.c. of *coli* bouillon, making a dilution of $\frac{1}{10,000,000}$. Tubes of neutral-red media were inoculated with amounts of this mixture varying from .5 to .01 c.c. In bouillon the reaction was present in 24 hours in all the tubes, and in glucose-agar where .5 and .25 c.c. were used. The same amounts of water taken from the carboy before the culture of *B. coli* was added gave no reaction. The results therefore corresponded to those of the previous experiment, an inhibitory action being apparent in the case of the glucose-agar tubes.

In a further experiment to each of the flasks E, F and G, Table I., 1 c.c. of a 24-hour old bouillon culture of *B. mesentericus* was added. Tubes of neutral-red media were then inoculated with 1 c.c. of this mixture and incubated at 37°, with the result shown in Table III.

TABLE III.

	FLASK E.	FLASK F.	FLASK G.
	Dilution $\frac{1}{10,000,000}$ <i>coli</i> bouillon $\frac{1}{1000}$ <i>mesentericus</i> „	Dilution $\frac{1}{100,000,000}$ <i>coli</i> bouillon $\frac{1}{1000}$ <i>mesentericus</i> „	Dilution $\frac{1}{1000,000,000}$ <i>coli</i> bouillon $\frac{1}{1000}$ <i>mesentericus</i> „
1 c.c. in neutral-red bouillon	In 24 hrs. fluorescence but little yellow In 48 hrs. full reaction	In 24 hrs. slight fluorescence below in some tubes. No reaction in one In 48 hrs. full reaction in all	In 24 hrs. no change In 48 hrs. reaction in two tubes In 72 hrs. reaction in all
1 c.c. in neutral-red glucose-agar	Coli reaction in 24 hrs.	Coli reaction after 72 hrs.	

The *Bacillus mesentericus*, therefore, when present in large excess, was able to delay the reaction considerably.

The laboratory tap-water was well adapted for estimating the value of neutral-red media as a test for minute quantities of *B. coli*. This water normally contains very few *coli* bacilli, and by the usual methods it is difficult to detect their presence. Thus of five agar plates, each containing .2 c.c. of water, only one showed a colony of *B. coli*. In neutral-red media the tap-water gave the following results:—

In bouillon :

- 2 c.c. Positive reaction in 24 hours.
- 1 c.c. Positive reaction in 24 hours in 40% of 15 examinations. No result in other cases.
- .5 c.c. Positive reaction in 14% of tubes. Delayed in one case till third day.
- .25 c.c. and below this. No reaction.

In glucose-agar :

- 2 c.c. No change on first day ; a few bubbles on second ; positive reaction on third or fourth day in every case.
- 1 c.c. Positive reaction in 33% of tubes, generally showing on fourth day.
- .5 c.c. One tube out of seven showed some reaction on the sixth day.
- .25 c.c. and below this. No reaction.

Where reactions were obtained with tap-water the presence of *B. coli* was on most occasions verified by making plates from the tubes. The colonies were then examined as to reaction and mode of growth on

various media, and in every case bacilli having all the characteristics of *B. coli* were demonstrated, save that the coagulation of milk was delayed in some cases till the fourth or fifth day, and was sometimes entirely absent.

In making the plates, more especially from glucose-agar tubes, it was remarkable how nearly pure were the cultures of *B. coli*. The yellow fluid found on the third day at the bottom of these tubes when 2 c.c. of tap-water had been used, was found generally to contain few other organisms.

As a qualitative means of detecting *B. coli* neutral-red media evidently offer great advantages owing to the large amount of water which can be examined. By the ordinary plate method minute quantities of *B. coli* are of course crowded out by other organisms.

I am deeply indebted to Dr W. Hunter, who planned the investigation and gave me much valuable assistance, and to Dr Bullock, in whose laboratory the research was made. A preliminary summary of the results was given by Dr Hunter in the *Lancet*, April 14, 1901, p. 1079.

CONCLUSIONS.

1. Neutral-red media afford a rapid and very delicate test of the presence of *Bacillus coli* in water.

2. By using varying quantities of water a rough estimate can be obtained of the number present, allowance being made for the influence of inhibiting organisms.

3. A negative result where a fair sample of water is examined may be taken as evidence of the absence of *Bacillus coli*.

4. Further investigation is needed to decide whether or not a positive reaction always indicates the presence of *Bacillus coli*; but as yet no case has been observed by the writer in which this bacillus was absent from a sample of water which gave a typical positive reaction.

NEUTRAL-RED IN THE ROUTINE BACTERIOLOGICAL EXAMINATION OF WATER¹.

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THE research which follows was undertaken with the object of determining how far the neutral-red reaction described by Rothberger could be utilised for the purpose of detecting *Bacillus coli* in water-supplies².

In accordance with Scheffler's recommendation I have used glucose media, broth or agar containing 0·5 % of glucose being employed throughout. With regard to the respective merits of broth and agar I am quite in accord with Rothberger, Scheffler, and Hunter that agar media (particularly agar shake-cultures) are best, but I find that in most cases excellent results can be obtained with broth. Glucose neutral-red broth was used in the routine water examination, but for testing individual organisms reliance was placed in preference upon glucose neutral-red agar shake-preparations. All incubations were performed at 37° C., and usually the reaction resulted in 24—48 hrs., but sometimes took several days if broth was used. It is not a matter of indifference what strength of glucose and of neutral-red is used. If neutral-red is added in excess the *B. coli* may not be able to reduce it, as is readily demonstrated by direct experiment. It was found that 0·1 c.c. of a 0·5 % watery solution of neutral-red (Grübler's) added to 10 c.c. of broth or agar gives the best results, and this was the strength employed.

The water was collected in small glass-stoppered bottles of about

¹ MS. received August, 1901.

² References to the literature of the neutral-red reaction have on the suggestion of the editors been omitted, as they are given in the preceding paper by Dr Makgill.

TABLE I.

Number	Kind of water	Number of organisms developing at 37° C.	Indol reaction applied to 5 c.c. of the water	Neutral-red test				Remarks
				Amount of water used	If reaction obtained	Time when obtained	If <i>B. coli</i> isolated	
I	Unfiltered public supply ...	47	+	50 c.c.	+	2 days	Yes	<i>B. coli</i> isolated from 2 c.c. grown anaerobically. The 50 c.c. not examined
II	" " " " " "	692	+	50 "	+	"	Yes	
III	A bore hole for a new supply ...	9	-	50 "	-	3 days	No	
IV	Unfiltered public supply ...	1	-	50 "	+	2 "	No	
V	A well ...	20	+	50 "	+	"	Yes	
VI	100 c.c. tap water + 1 c.c. sewage	2328	+	100 "	+	3 "	Yes	Isolated from $\frac{1}{1000}$ c.c. of the sewage
VII	" " + $\frac{1}{10}$ c.c. "	312	+	100 "	+	2 "	Yes	
VIII	" " + $\frac{1}{1000}$ c.c. "	55	+	100 "	+	3 "	Yes	
IX	" " + $\frac{1}{10000}$ c.c. "	31	+	100 "	+	3 "	Not examined	
X	Filtered public supply ...	3	-	100 "	-	24 hrs.	No	
XI	A contaminated brook ...	1370	+	6 "	+	"	Not examined	
XII	Same brook as XI after admixture of cemetery drains	1647	+	6 "	+	24 "	Not examined	
XIII	A ship's drinking water ...	18,080	+	2 "	+	3 days	Not examined	
XIV	A well ...	8	-	40 "	-	3 days	Not	Isolated from the 40 c.c.
XV	Unfiltered public supply ...	16	+	40 "	+	"	Yes	
XVI	" " " " " "	2	-	50 "	-	"	Not	
XVII	" " " " " "	18,000	+	2 "	+	24 hrs.	Yes	Isolated from the 2 c.c.
XVIII	Drinking water of a ship	1560	+	5 "	+	24 "	Yes	
XIX	Filtered public supply ...	1	-	40 "	+	48 "	Yes	Isolated from the 1 c.c.
XX	" " " " " "	0	+	40 "	-	4 days	Not	
XXI	Unfiltered public supply ...	37	+	40 "	+	48 hrs.	Yes	Isolated from the 40 c.c.
XXII	" " " " " "	33	+	40 "	+	48 "	Yes	

XXIII	Filtered public supply ...	2	102	+	5 c.c.	-	Not	An organism = <i>qq</i> isolated from the 10 c.c. which gives the neutral-red reaction
XXIV	" " " " " "	243	500 (about)	+	10 "	+	Not	also from the 10 c.c. and isolated from the 10 c.c. grown anaerobically in glucose formate broth
XXV	*Unfiltered public supply ...	about 10,000	Very numerous	-	10 "	+	Yes	Isolated from the 6 c.c.
XXVI	" " " " " "	9	150	+	6 "	+	Yes	The 40 c.c. examined
XXVII	A well ...	6	107	-	10 "	-	Not	
XXVIII	Unfiltered public supply ...	7	42	-	2 "	-	Not examined	
XXIX	Filtered public supply ...	80	192	+	30 "	-	Yes	Isolated from the 8 c.c.
XXX	Unfiltered public supply ...	210	370	+	40 "	+	Not examined	A very partial reaction only obtained with the 10 c.c.
XXXI	A suspected well ...	202	1120	+	2 "	+	Yes	Isolated from the 10 c.c.
XXXII	Filtered public supply. Same source as XXIV	7	88	+	24 hrs. 2 days	+	Yes	Isolated from the 40 c.c.
XXXIII	Filtered public supply ...	1	188	+	4 "	+	Yes	Isolated from the 40 c.c.
XXXIV	Unfiltered public supply ..	16	54	+	4-6 "	+	Yes	Isolated from the 40 c.c. An organism isolated = <i>pp</i> which gives a partial reaction
XXXV	" " " " " "	2	206	+	2 "	+	Not	<i>B. coli</i> not isolated, but <i>bb</i> isolated from the 5 c.c. which gives marked neutral-red reaction
XXXVI	A surface-contaminated well	1080	about 10,000	+	5 "	-	Yes	The 10 c.c. examined
XXXVII	A suspected well ...	4	254	+	24 hrs. 5 days	+	Yes	Isolated from the 10 c.c.
XXXVIII	Unfiltered public supply ...	191	620	+	10 "	+	Yes	Isolated from the 40 c.c.
				+	5 "	+	Yes	Isolated from the 5 c.c.
				+	3-5 "	+		
				+	2 "	+		
				+	4 "	+		

* Not packed in ice and only received day after collection, and so valueless for consideration of significance of *B. coli* in water and in relation to the numerical count.

TABLE I. (cont.)

Number	Kind of water	Number of organisms developing at 37° C.	Indol reaction added to 5 c.c. of the water	Neutral-red test				Remarks
				Amount of water used	If reaction obtained	Time when obtained	If <i>B. coli</i> isolated	
XXXIX	Unfiltered public supply ...	135	{	5 c.c. 40 "	+	2 days 4 "	Yes	Isolated from 5 c.c. grown anaerobically in glucose formate broth. Not found in the 5 c.c. glucose neutral-red broth
XL	A contaminated brook ...	1350	{	1 " 40 "	+	4 days 2 "	Yes	Isolated from the 1 c.c.
XLI	Unfiltered public supply ...	0	{	10 " 40 "	-	"	Not	The 40 c.c. examined for <i>B. coli</i>
XLII	A shallow well ...	140	{	10 " 40 "	+	3 days 5 "	Yes	Isolated from the 40 c.c. Could not be found in the 10 c.c.
XLIII	Unfiltered public supply ...	72	{	10 " 40 "	+	3 " 3 "	Yes	<i>B. coli</i> isolated from the 10 c.c.
XLIV	A contaminated brook ...	4500	{	5 " 40 "	+	24 hrs. 3 days	Yes	<i>B. coli</i> isolated from the 5 c.c.
XLV	Unfiltered public supply ...	136	{	10 " 40 "	+	2 " 3 "	Yes	<i>B. coli</i> isolated from the 10 c.c.
XLVI	Filtered public supply ...	9	{	10 " 40 "	+	3 " 3 "	Yes	<i>B. coli</i> isolated from the 10 c.c.
XLVII	Unfiltered public supply ...	1	{	10 " 40 "	-	"	Not	The 40 c.c. examined
XLVIII	Filtered public supply ...	192	{	10 " 40 "	+	3 days 8 "	Yes	<i>B. coli</i> isolated from the 10 c.c.
XLIX	Drinking water of a ship ...	84	{	5 " 40 "	+	4 " 2 "	Yes	Isolated from the 5 c.c.
L	Unfiltered public supply (water from a reservoir)	2100	{	10 " 40 "	+	24 hrs. 24 "	Yes	Isolated from the 10 c.c.

2 oz. (60 c.c.) capacity. After the different amounts required for the various steps of the routine examination were withdrawn, 10 c.c. or a smaller quantity of the water was added by sterile pipette to a tube of glucose neutral-red broth. To the remainder in the bottle, usually about 40 c.c., a second tube of 10 c.c. of glucose neutral-red broth was added. Both were incubated at 37° C. and examined daily. The exact amounts used varied a little, as can be seen from Table I. The liquid in the bottle usually took a longer time to develop the reaction than the more concentrated liquid in the test-tube. At first the neutral-red was added to the broth in batches subsequently to sterilization, but for the last ten to twelve waters the following modification was employed as preferable:—

The 10 c.c. or less of the water is added to the neutral-red broth as before, but instead of adding this *ordinary* glucose neutral-red broth to the remainder in the bottle the contents of a tube of *four times strength* glucose neutral-red broth is now added. Also the neutral-red is added to the broth before sterilization.

If the *B. coli* is present the mixture of broth and water becomes yellow and fluorescent.

Before the value of the reaction applied to detect *B. coli* in water can be affirmed there are obviously two questions which must as far as possible be answered. They are—

(1) If the *B. coli* is present will it always give this characteristic reaction?

And (2) Is the *B. coli* the only organism which may give this reaction under the conditions of the test?

To answer these questions and determine the value of the neutral-red test in routine water examination, fifty waters were systematically investigated bacteriologically. These waters were obtained from very varying sources, some from sources obviously polluted, others from suspected wells, springs, etc., while others were obtained from public water-supplies.

The answer to the first question can be most readily arrived at by considering the following:—

(a) In all the cases in which a negative reaction is obtained, is it impossible to find *B. coli*?

(b) Do all varieties of *B. coli* give the reaction in neutral-red broth?

(c) Are there any retarding or inimical agencies in waters which prevent the development of the reaction?

TABLE II.

Morphological and cultural characters of the B. coli isolated.

Water from which obtained	Broth (24 hrs. growth)	Milk	Indol	Gelatine slope	Motility	Gas production	Standard glucose neutral-red	
							Broth	Agar shake
I	Uniform turbidity no scum	Coagd. in 3 days	+	No liquefaction	Motile. Not active	+	Complete in 24 hrs.	Complete in 24 hrs.
II	" " "	" " "	+	"	Actively motile	+	" "	Yellow and fluor. in 2 days
V	Uniform turbidity scum	" " "	+	"	Motile. Not active	+	" 2 days	" "
VIII	Uniform turbidity slight scum	" 2 days	+	"	Sluggishly motile	+	" 3 days	Complete in 2 days
XV	" " "	" 3 days	+	"	Motile. Not active	+	" 24 hrs.	" "
XVII	Uniform turbidity no scum	" 6-7 "	+	"	Actively motile	+	" 3 days	Yellow and fluor. in 2 days
XVIII	" " "	" 2 days	+	"	Sluggishly motile	+	Marked fluorescence 2 days but remains red throughout	Fluorescence and yellow after 3 days
XIX	" " "	" 12 days	+ slight	"	" "	-	No change 6 days	A commencing yellow in upper layers only after several days
XXI	" " "	" 4 days	+	"	Motile. Fairly active	+	Fluorescence but red colour remains	Yellow and fluorescence only after several days
XXII	" " "	" 2 days	+	"	" "	+	Complete in 24 hrs.	Complete in 2 days
XXV	" " "	" 12 days	+	"	Sluggish motility	+	Marked fluorescence 2 days. Red colour remains	Complete in 3-4 days
XXVI	" " "	Not coagulated	+	"	" "	+	Marked fluorescence 2 days. Red colour remains	Yellow and fluorescence in 2 days

XXIX	Uniform turbidity no scum	Coagd. in 5 days	+	No liquefaction	Very actively motile	+	Complete in 2 days	Complete in 24 hrs.
XXXI	Uniform turbidity slight scum	" " 6 days	+	" "	Motile. Fairly active	+	" " 2 days	" " 2 days
XXXII	Uniform turbidity well-marked scum	" " 4-6 "	+	" "	Motile. Very slight	+	" " 24 hrs.	" " 24 hrs.
XXXIII	Uniform turbidity	" " 2 days	+	" "	Motile. Not marked	+	" " "	" " 2 days
XXXVI	Uniform turbidity thin scum	" " 11 days	+	" "	Sluggishly motile	+	" " "	" " "
XXXVII	" " no scum	" " 14 days	+	" "	No true motility	+	" " "	" " "
XXXVIII	" " "	" " 3 days	+	" "	Sluggish "	+	" " "	" " 3 days
XXXIX	Uniform turbidity	" " 3 days	+	" "	Actively motile	+	" " "	" " 24 hrs.
XL	Uniform turbidity slight scum	" " 8 days	+	" "	Motile. Fairly active	+	24 hrs. marked fluores- escence. Red colour remains throughout	Lower $\frac{1}{3}$ yellow and fluor- escent after 3-4 days' growth
XLII	" " "	" " ...	+	" "	Sluggishly motile	+	24 hrs. orange colour with marked fluor- escence	Complete in 2 days
XLIII	" " "	" " 7 days	+	" "	Non-motile	-	2 days no change, 4 days orange vel- low and fluorescent	A commencing orange in upper layers only after 4 days
XLIV	Uniform turbidity slight scum	" " 2 days	+	" "	Moderate motility	+	Complete in 24 hrs.	Complete in 2 days
XLV	Uniform turbidity no scum	" " 3 days	+	" "	Sluggishly motile	+	" " 2 days	" " "
XLVI	" " "	" " 4 days	- (10 days)	" "	" "	+	24 hrs. red colour marked fluores- cence. Remains red throughout	" " 4 days
XLVIII	Uniform turbidity slight scum	Not coagulated (8 days)	+	" "	Motile. Fairly active	+	Complete in 2 days	" " 2 days
XLIX	Uniform turbidity marked scum	" " ...	- (1 week)	" "	Actively motile	+	" " "	" " "
L	Uniform turbidity thick scum	" " ...	+	" "	Motile. Not marked	+	" " "	" " "

Taking these points in order.

(a) As can be seen from Table I., 11 waters gave a negative neutral-red reaction. Of these 10 were examined for *B. coli*. The method of examination is described below.

In none of the 10 waters examined could the *B. coli* be detected. The nearest approach to it was in Water XIX., in which a very partial reaction was obtained, and only after 4 days. From this water an organism was isolated which was probably a *B. coli* but which did not produce gas and gave no true neutral-red reaction when tested in pure culture.

(b) Hunter reports that all his *B. coli* gave the test. He however preferred agar cultures, and probably most of the *B. coli* he tested were not examined in neutral-red glucose broth. Scheffler found that all the *B. coli* excluding those organisms incapable of forming gas gave the reaction. Examining the *B. coli* isolated, I found that while the majority of them gave quite typical results with agar shake-cultures, several failed to give complete reactions with broth cultures. For details see Table II. It is noticeable that several gave delayed reactions, and in some the fluorescence disappeared or the red colour returned with time. Nos. XIX. and XLIII. gave very imperfect reactions with neutral-red. As can be seen from the table neither produced gas.

These results agree with Scheffler's in that the absence of gas-producing power was associated with unsatisfactory or absent neutral-red reactions.

(c) The reaction is essentially one of reduction, and it is by no means inconceivable that certain conditions, for example the antagonism of co-existing microbes, may prevent any *B. coli* actually present from producing this typical reaction. A thorough investigation of this question could not be made, but throughout the research it was steadily kept in view and a number of accessory experiments were made. The results obtained showed that given an equal start the *B. coli* will generally give the neutral-red reaction in glucose neutral-red broth and water, whether many other organisms are present or not, but that if the water organisms are incubated with neutral-red broth for several days and then the tube or flask is inoculated with *B. coli*, under these circumstances frequently, even usually, no reaction develops. Whether this is due to the *B. coli* not growing, or to the other organisms which have received a start preventing the reduction of the neutral-red, was not determined. Under

the conditions of the test as applied, there is probably very little danger that the other organisms present will prevent the development of the neutral-red reaction by any *B. coli* which are present in the water.

In answering the first question, therefore, the results obtained appear to justify the statement that a negative reaction, while not absolutely establishing the absence of *B. coli* in the water, yet makes its presence very improbable.

The attempt to answer the second question was made along the following lines. (a) By endeavouring to find the *B. coli* in all the waters in which a positive reaction was obtained, and (b) by endeavouring to find organisms in water other than the *B. coli* which give the reaction.

(a) Out of the 50 waters investigated 39 gave a positive reaction. Of these, 34 were specially examined for *B. coli*, and that organism was found in all but three. Of these three waters, in one no neutral-red reducing organisms were obtained, and probably the *B. coli* was present but was missed, while in the other two, organisms not *B. coli*, but which produced the typical reaction, were isolated.

The method adopted for isolating the *B. coli* consisted in brushing¹ the yellow mixture of neutral-red broth and water, usually much diluted, over a series of Petri dishes containing solidified agar. These were incubated at 37° C. for about 24 hrs. and then carefully examined. Usually only a few different kinds of colonies were present and in such cases all the kinds were subcultured and worked out, but where the varieties of colonies were many only those possibly *B. coli* were subcultivated. By incubating at 37° C. throughout, most of the water organisms are kept from growing, while the development of the *B. coli* is favoured. This method is very convenient though not especially delicate. In several cases fresh plates had to be brushed before the *B. coli* could be isolated.

(b) The reaction being one of reduction it was hardly to be expected that it would be specific. Indeed Rothberger has shown that

¹ For brushing plates the brusher which gives best results was made as follows. A fairly stout piece of flat indiarubber about $\frac{1}{16}$ th inch thick and $\frac{1}{2} \times \frac{3}{4}$ inch in area was fixed into a handle of wire such as is used to make diphtheria swabs. To fix the handle heat the end of the wire red-hot and hammer it flat and fix this into the indiarubber when hot. It readily burns its way into the rubber and when cold the melted indiarubber fixes it firmly. Such brushers can be easily, quickly and cheaply made, and can be sterilized repeatedly in the autoclave without damage. In brushing agar, or gelatine, they do not scratch the surface of the media.

the anaerobes, *B. tetani*, *B. anthracis symptomatici*, and *B. oedematis maligni* will change the colouring matter in the same way. Scheffler reports that he obtained the reaction with 3 out of 13 micro-organisms from spring and river water, and 8 out of 18 intestinal bacteria from man.

A large number of organisms were examined both from the waters which gave a positive reaction and from those which gave a negative reaction. With two exceptions (and one very slightly marked one) no organism other than the *B. coli* gave the reaction. It is important to remember that none of the organisms which would not grow at 37° C. were investigated; as under the conditions of the test they are not important. The neutral-red reacting organisms which are not *B. coli* are of considerable interest. No attempt was made to identify them, and here they are designated *qq*, *bb*, and *pp* respectively.

TABLE III.

	<i>bb</i>	<i>qq</i>	<i>pp</i>
Morphology	Short small bacilli	Short thick bacilli staining best at the ends	A larger bacillus which produces spores
Motility	Active	Very sluggish or nil	
Broth	Thick scum, broth not uniformly turbid	Uniform turbidity, thick scum	Broth clear with thick scum
Agar slope		Semitrans. growth with crinkled appearance	Opaque smooth white growth
Gelatine slope	White growth, rapid liquefaction	Very translucent bluish growth, slow liquefaction	White growth, fairly rapid liquefaction
Milk	Partial coagulation 2—3 days	No coagulation (1 week)	
Indol production	(7 days) No indol	(10 days) No indol	(7 days) No indol
Gas production	Nil	Nil	Nil
Glucose neutral-red broth	24 hrs. No change 48 hrs. Red colour and fluorescence 3 days. Quite yellow and fluorescent	24 and 48 hrs. No change. 3 days. Quite yellow and markedly fluorescent	24 hrs. to 4 days. No change. 5 days. Slight fluorescence. 7—10 days. Red colour remains but becomes markedly fluorescent 12 days. Fluorescent and orange colour
Glucose neutral-red agar shake	No gas and no fluorescence throughout. The only trace of a reaction is that the upper $\frac{1}{3}$ to $\frac{1}{2}$ gradually becomes orange in colour	No gas and no fluorescence throughout. The only trace of a reaction is that the upper $\frac{1}{3}$ of the agar becomes orange red (3—5 days)	No gas throughout. 2 days upper $\frac{1}{3}$ yellow and fluorescent, the rest red. The yellow part gradually extends until by 6th day it occupies the upper $\frac{1}{2}$

qq was obtained from Water XXIV., *bb* from XXXIV., and *pp* from XXXIII. In Waters XXIV. and XXXIV. no *B. coli* were found, but

in XXXIII. a typical *B. coli* was isolated in addition to *pp*. This latter organism was one which gave only a very partial and incomplete neutral-red reaction. Their characters as worked out are given in Table III.

From the above table it can be seen that these three organisms are perfectly distinct and that two of them, *i.e.* *bb* and *qq*, gave a complete reaction with neutral-red broth. All three however gave practically no reaction with glucose neutral-red agar shake-cultures. Both *bb* and *qq* were replated to ensure that they were pure cultivations.

From the results obtained, therefore, we cannot say that a positive neutral-red reaction can be taken as certain evidence of the presence of *B. coli*, but the latter was found in 31 out of 34 samples.

Leaving out no. IV., where the failure to find *B. coli* may fairly be ascribed to insufficient examination, we see that out of 44 waters examined by both methods 42 (*i.e.* over 95 %) gave successful results with neutral-red. In other words, if this reaction had been relied upon to detect the *B. coli* without subsequent isolation of the organism, the margin of error would have been less than 5 %, and this too when XXIV. is included which only gave a reaction after 7 days. In ordinary work XXIV. would certainly be excluded, and the margin of error for the samples examined would only be about 3 %.

It will be noticed that the number of positive results obtained is exceedingly high. The 50 waters consisted of the following classes:—

31 public supplies, 10 being filtered and 21 unfiltered.

8 wells, etc., many of which were suspected of being contaminated.

11 obviously contaminated waters.

TABLE IV.

Waters XVII., XXIV. and XXV. are omitted as they cannot be satisfactorily classed.

General character of water	Number of samples	Neutral-red reaction		<i>B. coli</i> looked for		<i>B. coli</i> found		Remarks
		+ reaction	- reaction	with + reaction	with - reaction	with + reaction	with - reaction	
Bad	25	25	0	20	0	20	0	{ not found with IV & XXXIV
Good	19	9	10	9	9	7	0	
Suspicious	3	2	1	2	1	2	0	
	47	36	11	31	10	29	0	

The details of these waters are shown in Table I. In Table IV. the waters are roughly classed into bad, good, and suspicious, this classification being based mainly on the result of their numerical count and in part on a knowledge of their source.

Of great interest are the waters in which the numerical count was satisfactory but which gave the neutral-red reaction. As can be seen from Table V. these were 9 in number and their chief features are reproduced in the table given below.

TABLE V.

Waters with satisfactory numerical count, but which yielded a positive neutral-red reaction.

Water	Number of organisms developing at		If <i>B. coli</i> found	Remarks
	37° C.	20° C.		
IV	1	42	Not	
XV	16	136	Yes	+ Reaction with 40 c.c., not with 5 c.c.
XIX	1	7	Yes	Only a very partial reaction and the <i>B. coli</i> isolated is not typical
XXVI	9	150	Yes	
XXXII	7	88	Yes	+ Reaction with 40 c.c., not with 10 c.c.
XXXIII	1	188	Yes	„ „ „ 40 „ „ „ 10 „
XXXIV	16	54	Not	bb a reacting organism, not <i>B. coli</i> , isolated
XXXVII	4	254	Yes	From a well suspected of being contaminated
XLVI	9	114	Yes	

With regard to these waters it is of interest to notice that XV., XIX. and XXXIII. were from the same public supply, XV. being the water from the reservoir near the gathering grounds and many miles from the town it supplies, XIX. the same water filtered, and XXXIII. the same water but collected from the tap of a neighbouring town supplied from the same source. Into the reservoir from which XV. was taken the only possible source of contamination is a small stream which is contaminated by a small inn on its banks and which runs into the reservoir. The same water was re-examined about a month later (XLI.) but then gave no neutral-red reaction and *B. coli* could not be detected. IV. was examined chemically at the same time and was found quite satisfactory. XLVI. and XXVI. gave satisfactory figures bacteriologically, but samples collected from the same sources, and at the same time, gave chemical evidence of contamination. Thus XLVI. gave free ammonia 0.0034, albuminoid ammonia 0.0174, chlorine 1.0 parts

per 100,000, and considerable sediment consisting of vegetable cells and debris. There was thus evidently marked vegetable contamination. XXVI. gave free ammonia 0.0512, albuminoid ammonia 0.0114, chlorine 1.5 parts per 100,000. Traces of phosphates present. Considerable deposit with vegetable debris and a few animalculae.

The extremely high proportion of positive results is puzzling and naturally arouses suspicions of contamination either in the application of the test or in the collection. With regard to the former especial effort was made to have all apparatus and media sterile, and control experiments were made from time to time. I think possible contamination at this stage may be excluded.

Contamination in collection may possibly have taken place in several instances, as a good many of the samples were collected by sanitary inspectors and others unskilled in collecting water for this purpose, so that the minute printed directions sent out with the sterile bottles may not have been accurately followed. On the other hand a considerable number of the samples were collected by myself and with the greatest care.

It is also necessary to remember that a good many of the specimens were from obviously contaminated sources, while a considerable number of the public supplies were known to be suspicious and several had been repeatedly condemned. Again, a number of them (7 of the 50 waters) were from one source—an unfiltered public supply—and a positive reaction was obtained in 6. This was a water supply in samples of which taken by myself (using glucose formate broth) I was able repeatedly to demonstrate *B. coli* in small quantities of the water. These facts in large part account for the high proportion of positive results obtained. Of the 50 waters only 31 were from quite distinct sources, the same supply having been sometimes examined separately in reservoir, tap, etc., or repeated.

I will only say in this paper that the results are somewhat surprising and tend to make me reconsider the significance of the presence of *B. coli* in water. The detection however of this organism in all the obviously bad waters points strongly to its association with contamination.

CONCLUSIONS.

(1) A positive neutral-red reaction obtained as above, while not absolutely diagnostic of *B. coli*, yet in the vast majority of cases points to the presence of that organism.

(2) A negative neutral-red reaction obtained as above does not certainly exclude *B. coli* but renders its presence highly improbable.

(3) The neutral-red test is very readily applied, and with reasonable care fallacies in its employment can be avoided.

(4) It is a test which is of great value in the routine examination of water.

STUDIES IN RELATION TO MALARIA.

II.

THE STRUCTURE AND BIOLOGY OF ANOPHELES

(*Anopheles maculipennis*).

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(*Continued from page 276.*)

BEFORE proceeding to describe the imago of *Anopheles*, we wish to record some observations regarding the hibernation of the larvae of various Culicidae. Grassi (1900, p. 47)¹ considered that *A. bifurcatus* hibernated chiefly in the larval form, for he found the larvae of this species in midwinter in Southern and Central Italy, the imagines being rarely encountered. It was quite different with *A. maculipennis*. In this case the imagines congregated in houses, huts, caverns, etc., whereas no larvae could be found during the winter. Though he assumed that it was so, his finding the larvae in midwinter in the first instance did not prove that they hibernated, for they might well have been derived from late-laid eggs. Ficalbi (1901, p. 66) says he has only seen the larvae of *A. bifurcatus* hibernate in Italy, but that during the past winter, which set in late, *A. maculipennis* was found later than usual

¹ See Bibliography, p. 75, this *Journal*, as also at the end of this paper.

along the Adriatic slopes. He thinks the larvae of this species may possibly live through the winter in warm countries like Sicily.

Of the larvae collected in various parts of England in the summer of 1900, only three lots hibernated in the laboratory. Whereas with the advance of winter all the larvae of *A. maculipennis* died off, those of *A. bifurcatus*, which were by no means as numerous, remained alive. One of us reported this observation in a preliminary note in June. As *A. bifurcatus* has likewise been proved to transmit the malarial parasite, it seems in place to make a note regarding its biology, especially as we have found that it may live for *seven to eight months* in the larval stage.

Observations on the Hibernation of A. bifurcatus larvae.

I. Larvae caught 12 Aug., 1900, at Gainsborough. Some lived until 12 April, 1901, *i.e.* eight months. The larvae were kept in tanks in a room which was used. None of the larvae pupated.

II. Larvae caught 10 Sept., 1900, on the river Ure. A number died during the winter. The first pupae appeared on 12 April, 1901, the imago appearing four days later. These larvae had lived seven months and eight days in the laboratory. Two others subsequently underwent successful metamorphosis.

III. Larvae caught 14 Sept., 1900, near Garstang. Of these one survived, pupating 19 March, 1901, the imago appearing eleven days later. In this case the larval stage had lasted seven months.

These observations prove conclusively that the larvae of this species are able to hibernate. Whether those of *A. maculipennis* are capable of hibernating or not cannot be positively stated, although it would appear unlikely judging from our observations and those of Grassi.

That the larvae of other species of Culicidae are capable of resisting a considerable degree of cold is proved by a number of independent observers. Gorham (15 Jan., 1901, p. 330) in the United States writes, "though ice of considerable thickness had formed above them, and they could breathe only the air collected beneath," larvae were found living in natural waters in December, 1900. Gorham does not state whether the larvae were those of *Culex* or *Anopheles*. Wright (13 April, 1901) states that he found *Anopheles* larvae (species not stated) hibernating beneath the ice at Torphins in Scotland. Annett and Dutton (27 April, 1901) in a preliminary note, state that *Anopheles* larvae (again species not stated) collected at Wye in December, had lived through the winter, but had not undergone metamorphosis when they published. Brakeley and Smith (June, 1901, cited by Howard, pp. 7 and 83—90)

of New Jersey, U.S., have made the interesting observation that the larvae of *Culex pungens* may hibernate in the water which is contained in the tubular leaves of *Sarracenia*. The larvae were found in January within the solid ice which was contained in the leaves, the temperature for some time having been 2 to 2.5° F. below zero (−19 to 20° C.). The larvae could be seen lying curled up in the ice. When the ice was thawed the larvae became active and fed, and on being transported to a room they slowly grew in size, the imago appearing in March. Smith suggests that the swarms of mosquitoes observable in Alaska and other northern countries may perhaps hibernate as larvae, maturing rapidly with the melting of the ice. The suggestion seems very reasonable.

Galli-Valerio and Narbel (27 June, 1901) have found the larvae of *Anopheles bifurcatus* in the vicinity of Lausanne in Switzerland, hibernating beneath the ice in January, February and March. The larvae which they collected, were placed in tubes, and the first imago was seen to appear in the end of March. The mean temperatures (Centigrade) during the three months were as follows:

January:	Mean	−0.5°	Minimum	−12.8°	Maximum	7.1°
February:	„	−2.84°	„	−14.6°	„	1.4°
March:	„	−2.73°	„	−6.7°	„	5.2°.

These observations regarding the resistance of culicid larvae to frost possess a considerable degree of interest: it is however essential that the species should be accurately determined in each case if the observation is to possess scientific value.

Observations on "stranded" Pupae.

When pupae are placed in shallow water or wriggle to the side of a saucer containing little water, they lie on one side breathing through but one respiratory trumpet. Five pupae of *A. maculipennis* were removed from the tanks within 3 to 48 hours of the time when they had issued from the larval skin, the pupae being carefully placed upon moistened filter-paper by means of a pipette and within test-tubes loosely plugged with cotton. All of these insects underwent successful metamorphosis within 48 to 72 hours, the temperature ranging between 20 and 23° C. Whenever the tubes containing the pupae were handled, the insects wriggled about vigorously, at times making a "long jump" to a distance of 1 to 2 cm. over the surface of the paper. Pupae of

various ages were next placed on dry filter-paper within tubes, but it was found that the majority shrivelled up and died. A few made unsuccessful efforts at extricating themselves, only succeeding in protruding the thorax; one fly extricated itself from the pupal-skin, but lost a leg in the operation, but in this case a little water had been allowed to flow on the filter-paper before the fly began to appear. It is evident then that the pupa cannot resist desiccation except to a moderate degree, metamorphosis not being impeded however by their simply being stranded on a moist surface. Experiments made with the pupae of *Culex pipiens* gave similar results.

Conditions which hasten Pupation and the Appearance of the Imago.

Howard (1901, p. 12) observed that when he placed creosote-oil on the water in breeding-jars containing *Culex stimulans* and *C. perturbans* larvae which were nearly full grown, their metamorphosis into pupae was hastened. The larvae struggled violently when the creosote was added, and, as they were about to transform, "the violent struggling evidently assisted in the breaking of the larval skin, leaving the pupa bare." Still more interesting was the observation that the pupal stage only lasted 15 hours instead of 48 hours. One of us was very much struck (26 July, 1901) by the effect of a showery afternoon on mature *Culex* larvae in a water-butt. The weather had been relatively dry for a couple of days before the downpour. After the rain had ceased a vast number of larvae were found to have become converted into pupae. The hastened metamorphosis was doubtless caused through the disturbance of the surface of the water.

The Emergence of the Perfect Fly.

When transformation is about to take place the pupa remains quietly at the surface of the water. Fine streaks due to the contained air appear upon the dorsum, extending antero-posteriorly between the respiratory trumpets, anteriorly as far as the base of the head. The air at first only extends a short distance posteriorly to the trumpets, but it gradually accumulates along the dorsal surface until it reaches to about the anterior margin of the seventh abdominal segment. Suddenly the pupa, which has become more buoyant, alters its position by extending its abdomen so that it comes to float parallel to the surface of the water, immediately beneath the surface-film to which it becomes attached. This usually lasts two minutes. Usually a minute later the dorsal

surface of the thorax protrudes through the dorsal slit in the pupa-skin and projects above the water. The insect's body seems to "grow" without visible effort out of the pupal covering, although a forward bend in the abdomen, near the thorax, indicates that the insect pushes itself out by pressing the abdomen against the pupa-skin. As the abdomen is protruded, the abdominal portion of the pupa-skin gradually fills with air. The head is pulled backward and then upward, and with the mouth-parts, palps and antennae, is then gradually pushed upward and forward, the appendages of the head remaining at first bent backward beneath the body of the insect. The base of the wings and the abdomen are gradually extruded, the wings are soon freed and immediately straighten out and harden. All this time the legs are still retained by their soft, folded ends which lie within the pupal covering, which also contains the posterior extremity of the abdomen. The insect now projects far beyond the anterior extremity of the pupa-skin, like the exaggerated figure-head of a vessel. If it were not for the air-filled pupa-skin which lies extended posteriorly, and is fixed to the surface-film, the fly would certainly fall forward into the water, for, taking the anterior extremity of the pupa-skin as the centre, the insect's head projects about as far anteriorly over the water as the pupal tail-flaps do posteriorly beneath the surface-film. We see in this straightening and filling of the abdominal portion of the pupa-skin with air, a very wise adaptation against the possible disaster of the tipping over of the fly into the water whilst issuing. We are not aware that the process has hitherto been closely watched or described. At this stage, then, the bunched legs all project backward and downward in a straight line until they enter the pupal covering. The front legs are first freed, the femoro-tibial articulation projecting forward and upward after the manner of a bent knee, the tarsal extremities still remaining in the pupa-skin. The posterior pairs of legs next bend backward and upward at the femoro-tibial joint. The tarsal extremities of the anterior legs are now withdrawn and all the tarsal joints rest flat upon the surface of the water. The appendages of the head now quickly assume their normal position. With the posterior extremity of the abdomen resting against the posterior margin of the slit in the pupa-skin, the fly now pulls the tarsal extremities of the posterior pair of legs forward, whereby the tibio-tarsal articulation is bent forward. The pupa-skin has now become quite filled with air. The abdomen is quite free, the tarsal joints of the second and third pair of legs are now withdrawn in rapid succession by short jerky pulls and the insect rests upon the surface

of the water, or partly upon the surrounding vegetation, or proceeds immediately to climb up the walls of the vessel in which it is contained. As stated above the insect seems to "grow" out of the pupa-skin, there being no visible effort until shortly after the wings have straightened, when a slightly forward and backward rocking of the body may be observed, followed subsequently by jerky movements when the extremities of the legs become freed. The straightening of the abdomen naturally facilitates its withdrawal. It is difficult to understand the mechanism whereby the insect leaves the pupa-skin unless it be by the pushing of the abdomen, but it is reasonable to suppose that the entrance of air into the pupa-skin plays no unimportant part in helping the insect to free itself.

In one case, the imago was seen to leave the pupa-skin in an exceptional manner, the pupa having floated into the corner of the rectangular aquarium. As the pupa straightened, its body was drawn by capillary attraction into contact with the walls of the vessel, so that it assumed a vertical position, which it maintained throughout the process of extrusion. As the pupa-skin filled with air, it was drawn more closely against the walls of the vessel, and the imago issued protruding backward over the surface of the water. As soon as its legs were freed it clambered up the sides of the aquarium.

If we study the empty pupa-skin we find it exceedingly buoyant, it being almost impossible to force it beneath the surface, owing to its containing air and offering a large surface for attachment to the surface-film. The dorsal slit, through which the insect issued, runs antero-posteriorly and is seen to be gaping, fusiform, and wider anteriorly at a point corresponding to that through which the bulk of the insect passed. The slit extends from a point corresponding to the anterior central margin of the imago's thorax, to a point corresponding to about the two hairs situated posteriorly to the respiratory trumpets as shown in Fig. 10, Plate II. Apparently the chitinous sack is in a state of tension just prior to the exit of the fly, so that the slightest exertion on the part of the insect causes the rupture of the pupa-skin, the slit widening of itself.

Wishing to preserve specimens of the insects in various stages of metamorphosis, we observed that they made violent efforts to extricate themselves when disturbed even during the first stage, when only the dorsal surface of the thorax protruded. Thus it was that some of them succeeded in a few moments in freeing themselves in a manner that otherwise took some minutes. It is essential that the surface of the

water shall not be disturbed whilst the fly issues from the pupa-skin, as even the slightest disturbance may be fatal. If the fly falls upon its back the dorsal surfaces of the wings adhere to the water and the insect is usually incapable of freeing itself. This exit of the fly is a very critical period in its life-history, and a number fail to free themselves. Reckoned from the moment when the thorax appeared, the fly usually freed itself completely, if left undisturbed, within 5 to 10 minutes. We cannot agree with some authors who state that the whole process takes but a few moments. A number of insects perish in the act of issuing from the pupa-skin: some die in the first stage, the head not being freed; and they may die, being unable to free themselves, at any stage following. Sometimes they make vain efforts to escape, only the tarsal extremities which remain within the skin keeping them back. We have seen them tear themselves loose at times, the violence of the effort resulting in the loss of one, two, or even, once, in the loss of three legs, which remained attached within the pupa-skin.

When the fly is left undisturbed, it remains resting until its limbs and wings have sufficiently hardened for it to fly and walk about with ease. It may take flight directly from the pupa-skin, or crawl to a safe place, where it remains quietly until the chitin hardens. The young imago is pale in colour, the thorax being pinkish-brown, the abdomen generally translucent and green, the legs pale brown, the wings only faintly showing the characteristic spots on their dorsal surface. The abdomen is at first very long: it may project 1 mm. beyond the wing-tips. Almost immediately after the insect has issued the abdomen begins to retract, this being accompanied by the expulsion of five or more very minute clear glistening greenish drops from the anus. The abdomen usually contracts to its normal size within about 5 minutes. When disturbed, the young insects were seen to fly perfectly in from 5 to 10 minutes after they issued. It was difficult to get them to fly sooner: if disturbed, they only moved away by means of their legs, or made unsuccessful efforts to fly, which ended in their falling into the water. The young imago begins to darken almost immediately, so that its colour changes within a few minutes. After 2 hours or more they acquire their normal coloration. It is difficult to say when complete hardening of the parts takes place. One insect was observed to rest in the characteristic position with the tips of the hind legs turned upward, 20 minutes after it had undergone metamorphosis. It was noticed that when the wings of the newly-hatched imago were cut off these did not become darkened.

It was repeatedly noticed that if anything prevented the insect from properly extending its limbs during metamorphosis or immediately after it had issued from the pupa-skin, the parts hardened and remained distorted throughout life.

ANATOMY OF THE IMAGO OF *ANOPHELES MACULIPENNIS* MEIGEN.

External Structures.

I. The Head.

The head of both sexes of *Anopheles* is shaped like a circular cushion, slightly flattened on its posterior surface, from the centre of which a membranous neck arises which unites it to the thorax. The surface of the head consists largely of two enormous, somewhat kidney-shaped eyes. These consist of several hundred ommatidia with their corresponding cuticular lenses very regularly arranged, with a beautiful greenish-black hue like so many emeralds set on a black ground. Dorsally the two eyes are almost in contact but are separated by a narrow area bearing a number of small grayish-black hairs which are directed forward and upward like a cock's comb. Ventrally the eyes are all but continuous, being separated only by a fine line (Fig. 16, Pl. IX.). In a transverse section passing near the posterior border of the eye the head seems to be almost completely surrounded by ommatidia and lenses. The posterior border of the eye is almost straight, but bends forward above to leave a triangular space, the vertex, which is gray in colour and bears numerous hairs and scales. The hairs are simple, black in colour, and a row of them is borne parallel to the posterior border of the eye and bending forward and overhanging the eye. These black hairs are also numerous at the sides of the head, but on the vertex they are replaced by certain flattened white scales, spatulate in shape. These latter, like the hairs, bend forward and overhang the head, giving the appearance of a rather ruffled, untidy head of hair. The anterior limits of the eyes are so shaped that they leave a triangular area on the front face of the head, one angle of which is ventral and two are latero-dorsal. From the former arises the clypeus overhanging the proboscis and from each of the latter an antenna.

Appendages of the Head.

In describing the appendages of the head we have adopted the nomenclature of Dimmock (1881). The appendages are as follows:—

1. A pair of antennae or feelers, markedly different in the male and female.

2. A pair of mandibles, absent in the male. These are the cultelli of some anatomists.

3. A pair of 1st maxillae each bearing a jointed palp. These are the scapella of some anatomists.

4. A pair of 2nd maxillae. These have fused together to form a median piece called the labium. They have no palp.

Besides these paired appendages there are three unpaired appendages connected with the mouth, (i) the median anterior labrum, with which according to Dimmock is united (ii) the epipharynx, and lastly (iii) the hypopharynx or tongue, a median style arising behind the mouth. In the following description we use the word proboscis to include the whole of the mouth-parts, viz. the mandibles, the 1st maxillae, the 2nd maxillae or labium, and the unpaired processes, the labrum, the epipharynx and the hypopharynx, with the exception of the maxillary palps, which take no part in biting. All these structures enter the skin except the labium, which acts as a sheath and guide to the other parts.

The antennae of the female *Anopheles maculipennis* consist of fifteen segments, the first two of which differ markedly from the succeeding ones. The first is an extremely short segment, just a ring of chitin (Fig. 13, Pl. IX.). The second segment is a flattened sphere in shape, attached at one side to the head and on the opposite side deeply pitted. From the centre of this pit the third segment takes its origin. The large swollen second segment lodges a special and highly remarkable auditory ganglion which will be described in connection with the brain. From it a nerve arises which traverses the antennae.

The third segment is markedly longer than any other, the fourth is the shortest: after the fourth the segments increase regularly in length, but the last six differ little if at all in that respect. The proximal end of each segment is colourless and transparent, and this colourless band comprises about a seventh of the total length of each segment, the rest of which is light brown. The transparent part bears in the female six fairly

long hairs set in a symmetrical manner with an angle of 60° between each, these curve forward like the ribs of an umbrella which has been turned inside out. The longest of these hairs measures about $\cdot 325$ mm., but they vary much in length (Fig. 5, Pl. IX.). Terminally each segment bears two short hairs also turned towards the tip, and the last segment, which ends in a blunt point, is protected by five such hairs. Other small hairs are borne on the segments, especially on those nearest the base, but they are not so definitely arranged nor so conspicuous as those mentioned above.

The antennae of the male differ markedly from those of the female, and their bushy nature is the most conspicuous naked-eye character by which the sexes may be determined. It is probable, as we shall see later, that it is by means of the sensory, probably auditory, function of these organs, that the male seeks out the female: at any rate the ganglion in the second segment or basal bulb is more highly developed and twice as large in the male as in the female, in correspondence with the increase in number and complexity of the antennal hairs.

There are sixteen segments, one more than in the female. The first is a mere chitinous ring, the second is similar to though larger than the corresponding part in the female. The third segment bears a few irregularly scattered hairs, the fourth to the fifteenth segment bear the long conspicuous hairs so characteristic of the male (Fig. 16, Pl. IX.). These hairs attain a length of $0\cdot 8$ or $0\cdot 9$ mm. Each whorl of hairs (there are twelve of them) is carried by two semicircular chitinous bars which clasp the segment of the antennae at an angle, each bar bears 25—30 hairs which spread out in a fan-like manner. The hairs of each half are not however in contact, so that two lines devoid of hairs, *i.e.* between the fans, run along each antenna. The hairs of the proximal segments are shorter than those of the distal, whilst those of each edge of each fan are usually shorter than the central hairs (Fig. 16). The hairs seem capable of considerable movement and at times the antennae seem spirally beset with hairs, but a closer inspection shows that in reality the hairs are arranged in half rings. The captive males, during the daytime, usually carried the hairs closely applied to the shafts of the antennae, the hairs being extended (as in Plate I.) when they began to fly about in the evening. On one occasion three males extended their antennal hairs after being fed on sugar and water, this being in the evening when they were active. One of them, on coming to rest, flattened down the hairs again with his fore-legs. The fifteenth segment is a very long one, four times as long as any of the others;

besides its proper ring it is covered with minute hairs pointing forward. The sixteenth and last segment is almost half the length of the penultimate, and covered with similar hairs: near its base it bears six long hairs like those of the female antennae, and it ends in a blunt point. The basal part of each segment is not transparent and thus the beaded appearance of the female antennae is not present.

Mouth appendages. The other appendages of the head are grouped together as mouth appendages.

Taking now the various mouth-parts in their morphological order, the mandibles are in the female extremely delicate, finer in fact than any other part of the apparatus. Each has the shape of a very long and narrow chitinous blade curved so that in cross-section it looks like a segment of a circle of some 60—70° (Fig. 9, Pl. IX.). Each is of a bright yellow colour and is solid chitin. At the base the mandible is slightly swollen, it has its origin from the sides of the labrum and we have detected no special muscle attached to its proximal end. The mandibles are moved by the complex of muscles which move the labrum. When the labrum is being pressed into the skin they also enter, being impelled by the same elastic force which induces the sharpened end of a piece of whalebone to pierce a soft body if the other end be pushed towards the surface. The distal end of the mandible is flattened and formed into a very fine knife-blade, something like a butcher's knife but rather broader (Fig. 3, Pl. IX.). The cutting edge of this is provided with 31 extremely fine teeth, very much finer than those of the maxillae and too fine to be completely shown in Fig. 1, Pl. IX. In the male the mandibles are absent.

The first maxillae are slightly longer than the mandibles, and although they also are extremely fine stylets they are considerably stouter than the mandibles. Each consists of a chitinous rod which on its outer surface passes into a very thin flange, so that in transverse section the maxilla has the outline of a razor hollowed on one side (Fig. 9, Pl. IX.). The flange is extremely fine and has the sharpest possible edge. With a very high power fine lines can be seen running obliquely across the flange and dividing it into polygonal areas which are longer than broad (Fig. 2, Pl. IX.).

At the tip of each maxilla the chitinous rod is produced into 13 minute teeth, though in comparison with the fine serrations of the mandibles they are coarse and blunt (Fig. 4, Pl. IX.). The maxillae arise at the side of the base of the labium (Fig. 12, Pl. IX.), and each bears on the outer angle of its point of origin a palp which is but very

slightly shorter than the labium and the other organs which form the proboscis. In life these palps are usually carried slightly above and external to the proboscis, and the three form as it were an almost equal limbed tripod. In diameter the maxillary palp is slightly, but only slightly, less than the whole complex which we here call the proboscis. When biting the palps are generally carried at right angles to the axis of the proboscis, well out of the way. In the female each palp is five-jointed, and as Figs. 1 and 11 show the relative length of the joints is not always constant. The first joint is very small, about as long as broad. The third joint is always the longest, then comes the second, then the fourth, and finally the last, which terminates in a simple rounded end. The whole palp is beset with scales, some finer, some coarser, those of the two basal joints are of the flattened, ribbed, scale-like variety, pointing towards the free end and overlapping one another.

In the male the maxillary palps differ from those of the female just described. The distal end of the third joint is enlarged and broadened, and the fourth and fifth joints are much broadened, flattened and spatulate (Fig. 16, Pl. IX.). Their inner surface and edges are provided with numerous stout and long hairs, and the flattened ribbed scales are more widely distributed along the outer surface of the organ than is the case in the female. These enlarged ends to the maxillary palps are visible to normal eyesight, and together with the antennae form a ready criterion of sex. In some males the spatulate extremity of the palp is turned outward so that it roughly resembles a hoe. When viewed from above the outwardly turned extremities are seen to bend off at about a right angle, the proboscis projecting between the diverging ends.

Attached to the base of the maxillae just at the point where the maxillary palps emerge between the clypeus and the base of the proboscis, and on the inside of the head, is a chitinous bar which runs backward towards the posterior ventral aspect of the head, to the chitinous covering of which it is attached by numerous muscles. This endoskeletal rod or apodeme is well shown in Figs. 11, 12 and 13. The rods from each side converge so that together they form a **V** whose apex is directed posteriorly. It is obvious that this rod and the muscles attached to it play a considerable part in the protrusion and retraction of the maxillae. The retractor muscles are the more considerable, but there are certain fibres which from their disposition would obviously serve to protract the maxillae and help them in piercing the skin.

It is perhaps worth while to recall here that the palps of the female

Culex are very short three- or four-jointed structures, in the male *Culex* they are however five-jointed and at least as long as in *Anopheles*, but in those we have examined the last joints are curved upward, and have not the strongly spatulate, flattened, and terminal joints of *Anopheles*. In *Aedes* the palps are short in both sexes.

The second maxillae are united to form a soft, dorsally grooved labium, the median and most ventral of all the mouth-parts. The labium is of fairly uniform diameter and has two joints only. The proximal is very long and covered with small scales which are replaced by hairs on the distal segment. This consists of two halves termed the labellae slightly separated in the middle line (Fig. 6, Pl. IX.). The complex of stylets which form the piercing organ of the mosquito are guided and directed between these two labellae much in the same way as a billiard-cue is guided between the first and second fingers of a player. The labium is covered by a thin coat of cuticle: its cavity is largely hollow but is traversed by nerves, two tracheae, and a certain number of muscle fibres. Within this space in those mosquitoes which are infested with the embryos of *Filaria bancrofti* and *F. immitis*¹ the nematodes, after leaving the muscles of the thorax or the Malpighian tubules, come for a time to rest. Two parasites are generally present at one time, and it would be interesting to know if these are male and female. How the nematodes leave the labium of the mosquito and enter the body of man is still a matter of conjecture. The labium certainly does not enter the skin.

All along the upper surface of this organ is the deep groove in which the piercing stylets are concealed; their relative position when at rest will be described after the consideration of two median structures which play a large part in the mosquito's bite. These are (i) the labrum, with which is combined according to Dimmock (ii) the epipharynx, and finally, (iii) the hypopharynx. The labrum-epipharynx is in cross section something like an Ω , the slit-like opening of the otherwise complete tube being ventrally placed (Figs. 9 and 10, Pl. IX.). The organ consists of a double layer of chitin with, except at the angles, practically no space between. At the base of the labrum-epipharynx these two cylinders which form its walls do however part company and a space appears between them. The more dorsal portion is continuous with the chitinous covering of the clypeus—that dorsal swelling at the root of the proboscis—the ventral unites along its edges and passes

¹ Noë. *Rend. Acc. Lincei*, Ser. v. x. 1900, p. 357; v. also Bancroft in *Nature*, LXIV. 1901, p. 416.

uninterruptedly into the chitinous lining of the stomodaeum (Figs. 8 and 11, Pl. IX.). In the space thus formed in the clypeus, numerous paired muscles pass from above downwards and from behind forwards. These raise the proboscis and doubtless help in the movements which insert and retract the stylets when the insect bites.

At its distal end the labrum is shaved down into a sharp point, which in the female runs smoothly to the tip, but in the male is truncated and rather like the tip of an Indian elephant's proboscis (Figs. 1 and 16, Pl. IX.). It is a characteristic criterion of sex. The slit between the incurved edges of the labrum is very narrow, so narrow as to almost form a closed tube. This is especially the case in the male. In the female the slit is closed by the apposition of the last stylet to be described, the hypopharynx.

In the female *Anopheles* the hypopharynx is like a two-edged sword. It has its origin just above the base of the labium and stretches down the labial groove, closing ventrally the slit-like aperture of the labrum-epipharynx. At its base the hypopharynx has its origin in a wedge-shaped chitinous mass pierced by the common salivary duct, and this basal piece is attached by two powerful muscles which run longitudinally along the ventral surface of the oral cavity to be inserted in a half-saucer shaped flange which projects laterally and ventrally from the chitinous stomodaeum (Fig. 20, Pl. IX.). These muscles doubtless serve to retract the hypopharynx.

We have compared the hypopharynx to a two-edged sword, but the thickened median portion of the sword lying between the two edges is more pronounced on the ventral surface. The dorsal surface which lies close against the labrum is flat or even very slightly curved. Along this ventral thickening runs the salivary duct to open at the pointed free end of the hypopharynx. It is a chitin-lined tube of extreme fineness, somewhere between .035 and .036 mm. in diameter, yet along it passes the cause of death and disease, ruining great cities and devastating large districts. Few ducts have played so large a part in the history of the world, or in the spread of civilization, for it is through this duct that the malarial parasites are expelled with the saliva of the insect in which they have undergone their development.

In the male the hypopharynx is fused with the labium (Fig. 10, Pl. IX.). As in the female it is traversed by the salivary duct, but it is no piercing organ, and for this reason the male cannot pierce the skin and bite.

We have termed the apparatus the component parts of which have

been just described the proboscis. The proboscis then is the biting organ, and consists of all the mouth-parts, labrum, mandibles, 1st maxillae, 2nd maxillae (labium), epipharynx united with labrum and the hypopharynx. The maxillary palps lie free from the proboscis, and although they doubtless play some part in selecting the spot to be bitten they take no actual part in the biting.

The arrangement when at rest of the various parts here enumerated is as follows. The labrum, which is soft and almost fleshy, is grooved along its upper surface, and in this groove lie the other organs of the proboscis (Figs. 9 and 10, Pl. IX.). Most dorsal of all is the labrum-epipharynx, forming a second groove whose opening is very narrow and faces downward. Immediately ventrally to this slit is the hypopharynx. Either by the approximation of the edges of the slit or by the pressing of the hypopharynx against this groove the labrum-epipharynx is converted into a tube which is continuous with the cavity of the mouth (Fig. 20, Pl. IX.); and along it, drawn up by the action of the suctorial pharynx in the head, the liquid food, whether vegetable sap or animal blood, is drawn. At the angles of the labrum lie the two mandibles: the inner end of them is between the labrum and the hypopharynx, and in this respect they differ in position from those of *Culex*, where according to Dimmock the mandibles lie ventral to the hypopharynx between it and the 1st maxillae. Below the hypopharynx lie the maxillae, very neatly fitting in each side against the thickening formed by the salivary duct (Fig. 9, Pl. IX.). In the male the mandibles are absent: the 1st maxillae lie at the angle of the labrum; and the hypopharynx is as described above fused with the labium.

Method of feeding.

The newly-hatched insect is unable to perforate the skin until the mouth-parts have hardened, a process which requires a variable length of time. Gray (1900, p. 583) of St Lucia has seen Culicidae suck blood six hours after transformation. We have seen *A. maculipennis* feed readily after 24 hours, so that the statement made by Creagh (1899, *Brit. Med. Journ.*, 1. p. 1062) that the insects will not feed until after 6—8 days is wrong. Kerschbaumer (1901) thinks he has observed the imago drinking water whilst issuing from the pupal skin, and that the insect's abdomen became thicker. We feel sure that he is in error. To begin with, the insect has all it can do to free itself. We

have repeatedly observed the insect issuing, watching the process from the side with a magnifying-glass, through an aquarium made of thin glass. We do not doubt but that Kerschbaumer was deceived through watching the process from above. When watched from the side, the insect is seen to keep its mouth-parts away from the surface of the water, and although it may occasionally occur that they come in gentle contact with the surface-film, this does not mean that the imago is drinking, but that it has overbalanced itself. The abdomen as we have already described shortens rapidly and therefore may appear somewhat thicker after completed transformation.

When about to feed, both sexes move their antennae and palps out of the way, that is upwards, so that they form nearly a right angle to the axis of the mouth-parts which are inserted into the skin (Pl. X.).

When feeding on fluids or the moist surface of fruit, the insects feel about with the two-lobed extremity of the labium, and having found a suitable spot proceed to use their suctorial apparatus.

The males feed more slowly than females and take up very much less nutriment. When five males and five females were allowed to feed on milk and sugar, the females had gorged themselves on an average within 5 minutes, whereas the males took an average of 8 minutes to complete a very frugal meal, only the anterior two-thirds of their abdomen being slightly distended. And it was moreover noticed that the males evidently exerted themselves more in feeding, the sucking being accompanied by rapid to and fro movements of the palps and antennae, an appearance not observable when the female was feeding. When, as in blood-sucking, the skin has to be pierced, the female proceeds to use her stylets, which are worked to and fro with a visible effort which is made evident through slight forward and backward and lateral movements of the head. If it does not succeed in obtaining blood from the puncture, it gradually withdraws the stylets and tries again elsewhere. In one case one of us observed a fly try four times before it succeeded in obtaining blood. The blood is drawn up in the hollow of the labrum-epipharynx, a toxic saliva being injected through the delicate tubular, sharply pointed hypopharynx. The labium does not penetrate the skin. Its lobes rest upon the surface at the point where the stylets enter, and serve to steady and direct them. In sucking blood, the labrum-epipharynx, mandibles, maxillae and hypopharynx penetrate the skin to a greater or less depth until blood is obtained. They may penetrate almost to their entire length. As these organs penetrate the labium becomes bent backwards like a bow. If the

mouth-parts penetrate deeply, the labium becomes bent at an angle, the upper portion, which is shortest, being directed backward, the lower forward, forming two sides of a triangle which is closed by the stylets, which penetrate the skin at the point where the two lobes of the labium are resting. When the parts have penetrated, saliva immediately issues from the aperture of the hypopharynx. It has been assumed that the saliva is injected with the object of preventing the coagulation of the blood whilst it ascends through the groove of the labrum-epipharynx. That the saliva injection precedes and accompanies the act of blood-sucking was made very evident in the observation above referred to where an insect penetrated the skin at several places. Ross, Annett and Austin (1900, p. 24) have made the same observation. All the points of puncture reacted by becoming swollen and itching; the one from which blood was obtained reacted most, consequent upon the insect injecting more saliva than at the other points. Where the skin is thick and resistant the stylets are held almost at a right-angle to the long axis of the body, otherwise they project slightly forward. A detailed description of the internal mechanism by which the insect sucks blood is reserved for a future communication, in which the internal anatomy of the fly will be duly considered.

When the female has filled herself, the process when undisturbed lasting 2 to 3½ minutes by the watch, she withdraws her mouth-parts by bracing the legs against the skin and raising the body.

Tubular Tunnels.

A curious feature of the head of *Anopheles* is that it is pierced in an antero-posterior direction by two tubes (Fig. 13, Pl. IX.). These tubes open in front by a slit-like trumpet-shaped orifice situated between the anterior limit of the eye and the side of the clypeus: then converging slightly they pass backward and open by two trumpet-shaped apertures into a slight depression at the back of the head, near the ventral edge, below the neck. The tubes thus form a complete passage through the head, through which a bristle could be passed if it were fine enough, and through which air doubtless streams as the insect flies about. The tubes are lined by a well-marked layer of chitin which shows none of the spiral thickening associated with the tracheae. In its course through the head each tube is not of uniform diameter, and half-way along it bears a sharp, dorsally projecting spine, and near the posterior end it sends off another dorsally placed process which fuses with the

chitinous back of the head. This acts as a stay. The tubes lie in the angle formed by the great optic ganglion with the brain: they undoubtedly serve as a kind of strut to strengthen the chitinous exoskeleton of the head and to prevent its exoskeleton from collapsing. They also serve for the attachment of muscles, the chief of which are loose aggregates of more or less isolated muscle fibres—rather like those of a leech—which run to the maxillary palp. Other muscles run to the first sucking pharynx and several to the antennae.

Mr D. Sharp tells us that he has observed somewhat similar tubes piercing the head of the Sialid, *Corydalis*, and very similar tunnels are described by Miall and Hammond (1900, pp. 89—90) in the head of *Chironomus dorsalis*. The last named authors also mention and figure (Fig. 62) their occurrence in *Anopheles maculipennis*. Their suggestion that the tunnels are of the nature of apodemes which remain hollow seems probable.

The Neck.

The greater part of the wall of the neck is soft and only very thinly chitinised, thus allowing considerable mobility to the head. It is however strengthened by two lateral chitinous plates which approach one another dorsally but do not fuse (Fig. 15, Pl. IX.). Underneath, these two halves approximate, but do not unite, by means of a strap of chitin which extends across the neck like the two ends of a stand-up collar. The whole forms a skeleton roughly resembling an H broken in the middle. From the anterior inner angles of this sclerite two stout chitinous bars run forward towards the head. Posteriorly these are jointed on to the H shaped piece. They serve for the attachment of muscles. The remaining integument of the neck is delicately papillated and is transparent, as is shown by the red spot which appears there in the insect when sucking blood (Fig. A and Plate X.).

II. The Thorax.

Of the three divisions into which the body of an insect is primarily divided, the head, the thorax, and the abdomen, in *Anopheles* the thorax is by far the largest. It is perhaps some 12—15 times as big as the head and 4—6 times as big as the abdomen. The latter is however very extensile and when distended with eggs or by a heavy meal these proportions are altered.

The thorax is, roughly speaking, the shape of a four-sided pyramid whose lateral sides are twice as long as the anterior and posterior sides are broad. The apex, which looks ventralwards, is cut short, and here the three pair of legs emerge. The base of the pyramid, which is the dorsal surface, is strongly arched both from before backward and from side to side. Looked at from above, the angles of the base of the elongated pyramid are seen to be slightly shaved off so that the dorsal surface seems eight-sided; but of these sides the lateral are by far the largest. Anteriorly the thorax protrudes over the retracted head, posteriorly over the anterior limit of the abdomen.

The homologies and the limits of the sclerites which make up the skeleton of the thorax are very difficult to determine. Wherever possible the nomenclature of Brauer has been followed.

The dorsal surface is then a broad, slightly arched plane corresponding with the praescutum and scutum of Brauer. This is of a grayish-brown colour and hairy. Besides the smaller hairs there are a number of longer bristles symmetrically placed, and these are especially prominent towards the sides.

Near the posterior limit of the dorsal surface, between and just behind the insertion of the wings, is a curved thickening of the integument which arches over the back and serves to strengthen the exoskeleton in this important region of the body: this is the scutellum. It bears very conspicuous rows of hairs. Behind comes a rounded, vaulted, triangular sclerite, the post-scutellum, which reaches almost to the first abdominal segment.

The portion which we have here, following Brauer, described as post-scutellum is by Theobald (1901) called the metathorax. It is an important region, as the presence or absence of hairs and scales on it is a matter of generic importance. In *Anopheles maculipennis* it is, as Theobald says, nude.

According to Brauer all these dorsal pieces are part of the mesothorax; the prothorax is confined to the anterior surface of the thoracic pyramid and does not appear on the upper surface at all (Figs. A and 15, Pl. IX.). The metathorax is similarly almost squeezed out dorsally between the post-scutellum and the first abdominal segment, and appears only laterally and ventrally. There appears to be a trace of the metanotum or dorsal portion of the metathorax, forming a very narrow band between the post-scutellum and the first abdominal tergum.

Ventrally the neck passes into the thorax, and its transparent integument is extended on to the median area of the prothorax. This

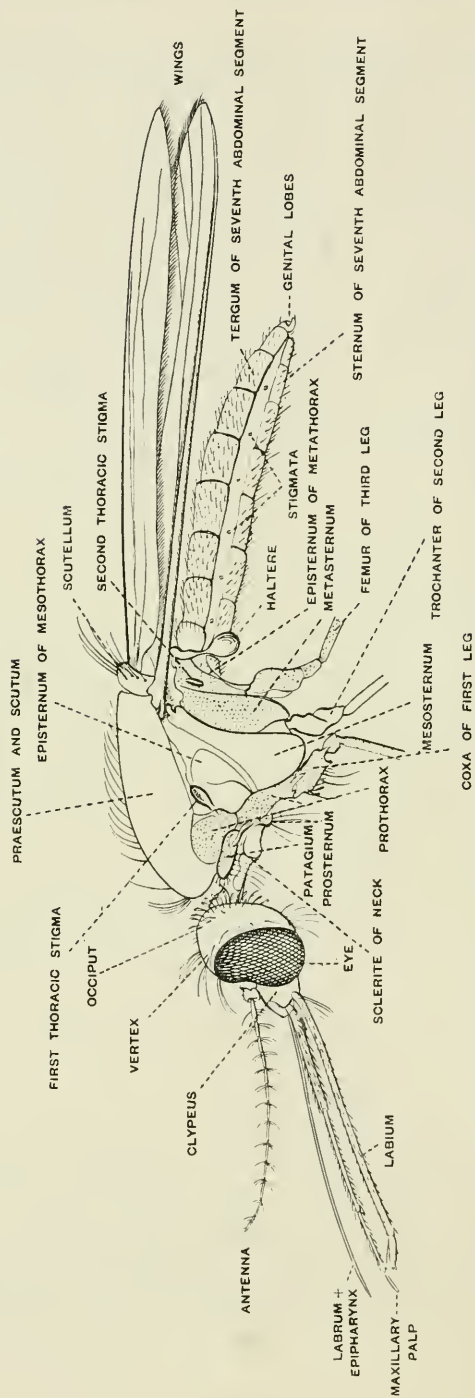


FIG. A. Side view of a female *Anopheles maculipennis* Meigen \times about 22 to show the various parts of the body. The prothorax and metathorax with their respective legs are dotted.

is very manifest when the female mosquito is sucking blood, a bright red bead appearing here and contrasting with the otherwise sombre hue of the thorax. This area is bounded posteriorly by the origin of the prothoracic or first pair of legs, and laterally by a pair of plates bearing a pair of sausage-shaped organs called the patagia (*v. p.* 477). Between each patagium and the origin of the leg is a small prominence bearing particularly large hairs. The same plate of exoskeleton which bears the patagium extends along the side of the body almost as far as the anterior border of the first or mesothoracic spiracle. This together with another piece is figured and described by Christophers (March, 1901, plate I.) as the pleuron of the mesothorax. We regard it as prothoracic and have so shaded it. Sharp (1895, p. 446) indeed regards the first spiracle of the Diptera as prothoracic. Whichever segment it really belongs to it is the largest spiracle in the body and opens by a wide-mouthed cavernous-looking orifice. This prothoracic portion of the lateral walls of the body is slightly hollowed out like a sunken cheek.

The sides of the mesothorax form a large part of the side of the thorax. Above in the region of the spiracle is an area which appears to correspond with Brauer's episternum, and below, not very clearly marked off from the episternum, is the mesosternum. This portion is greatly enlarged and stretches ventrally to form the apex of the pyramidal thorax. Dorsally it runs up to the insertion of the wing, where it is raised above the surface into a tri-lobed knob. It looks as if this knob fitted into a socket at the base of the wing when the wings are extended at right angles to the body.

The metasternum of the third or post-thoracic segment is similarly enlarged, like that of the second segment, in connection with the attachment of muscles: between the two the second pair of legs emerge: close behind the last arise the third pair of legs. Above and slightly behind this is a region, the episternum (?) of the metathorax, which bears the second thoracic spiracle and from which the halteres arise.

Behind and between the last pair of legs and the anterior abdominal segment is a somewhat triangular patch of soft and rather transparent integument.

On the ventral surface the following sclerites occur. Behind the neck, like a collar of a coat, two prosterna (Fig. 15, Pl. IX.). In the middle line these meet in a straight suture. Laterally these prosterna bear the knob of hairs already mentioned. The cords from the patagia, to be mentioned hereafter, run into the lateral angles of the prosterna.

Between the two prosterna and the coxa of the first leg is a region of soft integument which permits the movement of the coxa as the leather joints permit movement in plate-armour.

Owing to the great bulk of the mesosterna the interval between the first and second pair of legs is greater than between the second and third pair: in fact the rounded lobes of the mesosterna almost meet in the middle, and the two are strongly 'tied' together by a transverse bar or bridge of thick chitin, which forms a very conspicuous and striking structure when the legs are removed (Fig. 17, Pl. IX.). The swollen lobes of the metasterna do not approach each other so closely, but end external to the insertion of the last two pair of legs. The mesothoracic legs emerge from a plate which has a line or groove in the median line: this plate must belong to the mesosternum (Fig. 17, Pl. IX.).

The Legs.

The six legs are each composed of the typical Insect parts, viz., the coxa, trochanter, femur, tibia, tarsus, the last of which is built of five segments and ends in a pair of claws. The six coxae or basal joints of the three pairs of legs converge about the apex of the pyramidal thorax: they all slope inwards and downwards. The coxa of the first leg obviously arises from the prothorax. It is closely adpressed against the anterior surface of the mesothorax. The coxa of the mesothoracic leg is wedged in between the mesothoracic and metathoracic mesosterna, to the posterior surface of which the coxae of the hind or metathoracic legs are applied. The right and left coxae of these last-named limbs touch each other, and are even for a short distance on their hinder aspect fused together. All three pairs of coxae are short, cylindrical pieces bearing many hairs: particularly is this the case on the anterior face of the first pair. Distally each bears the second joint or trochanter, a piece shaped like a signet-ring, broader on one side than the other. The measurement of the coxa is difficult, as it is of irregular shape, but roughly speaking it is $\cdot 5$ mm.; the trochanter is from $\cdot 1$ to $\cdot 2$ mm.

The remaining segments of the leg are the femur, the tibia and the five-jointed tarsus. The length of these various segments is far from constant in different specimens.

Each segment of the leg is covered with numbers of the flattened,

longitudinally striated scales which have been described in many parts of the body. They vary somewhat in shape, but as a rule are something like old-fashioned cricket-bats in outline. Towards the distal end each segment swells out, and here there are longish hairs as well as scales that overhang the joint. The metatarsus, as the first segment of the tarsus is called, bears a double row of little black spines unlike anything on the other segments of the legs.

In the male the terminal segment of the first pair of tarsi is hollowed out on one side like a spoon: this area bears a number of curved short hairs with enlarged bases which encircle three-quarters of the hair like a collar and permit movement in one plane only (Fig. 18, Pl. IX.). The anterior leg in this sex terminates in a peculiar claw, which at its base bears two short, curved, lateral stumps of unequal size and is then continued into a long hook which forks into two in a plane at right angles to that in which the two stump-like processes lie. Both the hind legs bear two simple hooks, adjacent or fused at their bases, and divaricating at their tips. In these limbs the terminal segment is not hollowed but is cylindrical throughout (Fig. 19, Pl. IX.). Some authors write as though the front legs bore—like the others—two hooks. We have only observed one, though in some cases there seems to be a slight suture between the main hook and the lateral stump-like processes. Theobald (1901) states that the “fore and middle” claws are “always unequal” in the male, but in our specimens of the *A. maculipennis* it was with one exception the anterior claws only that departed from the ordinary structure of the other legs and of those of the female. In one specimen we noticed that it was the second pair of legs which had the hollow terminal tarsal segment and the forked hook, and in this case the hooks of the first pair of legs resembled those of the third. In the female all the tarsi terminate in the double hook, and the terminal joint is not hollowed out in the manner of the terminal tarsal segment of the prothoracic leg of the male.

The single muscle which bends the hooks is attached to a short chitinous piece, the “Streckplatte” of de Meijere (1901) and the extensor-plate of Packard (1898), which in its turn is attached to the hook. The hook bends on a joint which seems to be borne by a chitinous thickening of the rim of the end of the last tarsal segment. It straightens itself by elasticity. The empodium, a median process, which in many insects projects between the hooks, is possibly here represented by a short process bearing a tuft of hairs (Fig. 18, Pl. IX.).

The Wings.

The wings arise from high up on the sides of the mesothorax between the scutum and the episternum of that region. Their point of origin is a longitudinal slit, the posterior limit of which is at the level of the scutellum. The base of each wing is overhung by a series of stout hairs which arise from the scutum.

The anterior margin of the wing is straight and there are no lobes or indentations, but near the base of the wing the posterior edge is indented twice, and two lobes are thus produced which we may follow Sharp (1895) and call the squama and alula (Fig. 21, Pl. IX.). The squama is the most proximal portion; when the wing is extended the squama forms as it were a hollow vault or armpit, and the large hairs which project backward from its posterior edge form with certain hairs which arise from the metasterna a hollow dome. It is the squama which, when the wing is at rest, is thrown into a kind of fold, such as is made if the end of a strip of paper being fixed the paper be twisted through a right angle.

The alula is hardly so much a lobe as a depression: it lies as it were sunk within the margin: the depressions which limit it are deep, and its edge is but slightly rounded. This edge bears a sparse fringe of small hairs which are continued on to the anal or basal angle of the wing. These hairs continue along the edge of the wing but are soon replaced by the characteristic scales which fringe the posterior margin.

The area of the wing which is bounded behind by the squama and alula is broken up by a series of thickenings and ridges, from some of which the nervures take their origin. These ridges are not constant, they differ markedly in different specimens, those figured (Fig. 21, Pl. IX.) have perhaps the average arrangement. The ridges on this portion are probably formed when the wing loses its moisture and dries up, and the variety in its pattern is doubtless due to the varying activities of the many factors which play a part in this process.

Amongst these irregularities on the under surface of the base of the wing, in the axilla as it were, is a small socket which very accurately fits on to a knob borne by the episternum of the mesothorax. When the wing is in use this arrangement doubtless serves to maintain the forward position of the wings.

Nervuration of Wing.

In mapping out the nervures of the wing we have followed the scheme of Comstock and Needham (1898), in whose recent attempt to reduce the nervuration of insects to a system there is an account of the various views held by other entomologists. The accompanying figure (B) shows that the nervures of *Anopheles* correspond essentially with those figured by Comstock and Needham for *Dixa*, the chief differences being the much greater extension of the subcosta in *Anopheles*, the failure of the second radius and combined fourth and fifth radii to be connected with the first, and the presence of the cross-nervure *O*.

From the diagram it will be seen that each nervure is named after the main branch from which it arises, and the different branches are distinguished by the numerals 1, 2, 3, etc. beginning with that lying next to the anterior edge of the wing. When two nervures have coalesced the resulting nervure is indicated by such a symbol as R_{4+5} which indicates that the fourth and fifth radii have fused. The various cells or areas into which the wing is divided are in this scheme named after the chief nervure which takes part in forming its anterior limit.

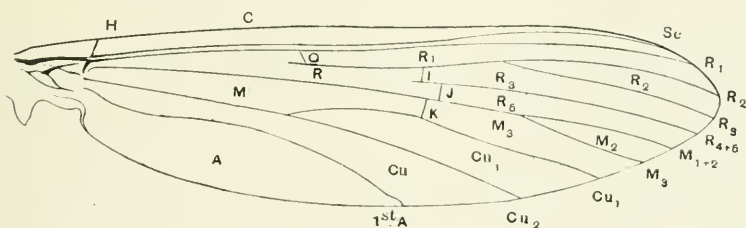


FIG. B. The right wing of a male *Anopheles maculipennis* Meigen \times about 14.

The scales have been removed to show the nervuration.

- A. Anal area. 1st A. Anal nervure. C. Costa. Cu. Cubitus. H. Humeral cross-nervure. I. Cross-nervure between R_1 and R_{4+5} . J. Cross-nervure between radial and medial systems. K. Cross-nervure between medial and cubital systems. M. Media. O. Cross-nervure between R_1 and R_2 . R. Radius. Sc. Subcosta.

The cross-nervures connecting the longitudinal ones still remain to be mentioned. These are the humeral (*H*, Fig. B) running from the costa to the subcosta, a very constant cross-nervure; the radio-medial (*J*, Fig. B) connecting the system of the radius with that of the medius, and the medio-cubital (*K*, Fig. B) uniting the medial system with that of the cubitus. These three are very generally met with, but there is

in *Anopheles* a fourth cross-nervure uniting R_1 with R_{4+5} (I, Fig. B), this exists also, but is much smaller in *Dixa*, and a cross-nervure (O, Fig. B) which unites R_1 with R_2 . This last seems hitherto to have escaped notice.

As a rule the wing of a dipterous insect is divided into two areas of nervures by an interval which is traversed but by one short transverse bar. This bar in the case of *Anopheles* appears to be the radio-medial.

All the veins are covered with scales, those on the upper surface being of a pointed feather shape, and those on the under surface more usually truncated. Along each nervure these scales are arranged in a feathered series, all pointing to the outer edge of the wing. The spots or maculations on the wings are caused by an unusual aggregation of these scales in certain places (Fig. 21, Pl. IX.). These are four in number in *A. maculipennis*, (i) at the point of origin of the radius nervure, which as is just stated arises suddenly in the middle of the wing; (ii) at the point of origin of the R_{4+5} , which has a similar origin; this spot can with the aid of a single lens be resolved into three because the aggregation of scales on the R_{4+5} seems to have set up by sympathy an aggregation at the same level on the adjacent medius and cubitus; (iii) at the point where the R_1 forks into R_2 and R_3 ; and (iv) at the point where the M_3 forks into M_{1+2} and M_3 .

Along each nervure there is usually a double, sometimes a treble row of the striated scales which spread out right and left.

One of the most beautiful structures in a mosquito is the posterior edge of the wing. Along its upper edge it bears a row of scales which attain their greatest length in the region next the alula. These lie side by side or even slightly overlapping. They stand out from the edge like a regular row of cypress-trees (Fig. 14, Pl. IX.). Alternating with these and arising from the other or under side of the wing is a second series of pointed scales about half the size of the others. Along the upper edge of the wing close to its margin is a definite ridge, and this ridge bears opposite every alternate big scale a small, somewhat battledore-shaped scale which hangs obliquely over the point of insertion of the big scale. The whole forms a structure of singular regularity and beauty. It tends to disappear along the outermost edge of the wing, and there passes with no abrupt change into the anterior border, where the scales are massed together and overhang one another in a matted and irregular way.

The greater part of the tissue of the wings is transparent. It bears, as is shown in Figs. 14 and 21, Pl. IX., a number of small spines like the

skin of a shark, but these diminish but little the transparency of the membrane. They however seem to break up the light, as it is this part which gives rise to the beautiful iridescence of the mosquito's wing.

The size of the wings varies considerably in different females. Out of a series of five the largest measured from the external tip of the wing to the groove between the alula and the squama 6 mm. in length and 1.5 mm. in breadth at its greatest. The smallest wing of the same series measuring the same area was 4.8 mm. \times 1.3 mm. In each case the measurements do not include the scales. The male wings are smaller. Of a similar series of five the largest measured 5 mm. \times 1.1 mm. and the smallest 4.3 mm. \times .9 mm. It is thus evident that the male wing is more slender than that of the female.

The Halteres.

The Halteres or Balancers arise from the metathorax close to the junction of the metathoracic episternum with the post scutellum. They are usually regarded as the representatives of the metathoracic or second pair of wings, which are always absent in the Diptera but are found in nearly all other insects. The halteres arise from a broad base which quickly narrows, forming a small cone, from the apex of which a short rod arises. This chitinous rod is not circular in section but slightly irregular: proximally it is jointed and is capable of a certain amount of movement, twitching up and down when flying. At the outer end the chitinous rod expands into a rounded knob. The basal half of the knob has a thicker chitinous covering than the distal, which is enclosed by a very thin membrane: around the equator of the knob lie a number of small scales which overhang the thinner portion to a slight extent. The distal hemisphere is itself studded with small brown bodies probably of a sensory nature. In the majority of preserved specimens this outer half does not bulge out, forming a club-shaped end to the haltere, as it should in life, but is sunk in or invaginated.

The whole organ is about .4 mm. in length. When the wings are folded the halteres usually lie backward, overhanging the first abdominal segment, and their tip reaches to the posterior edge of this tergum. When the wings are in a position of flight the halteres usually project out from the body, not quite at right angles but pointing backwards.

The Patagia.

On the anterior face of each side of the prothorax, in front of the first pair of spiracles, is a sausage-shaped body, termed by Christophers

the patagium, resting on the shoulders of the mosquito like a pair of epaulettes. It corresponds in position very fairly with the patagium of the Lepidoptera and of some other insects (Cholodkovsky, 1896 and 1897). The structure in *Anopheles* resembles very much the shape of a short sausage, applied to the prothorax. It is separated by a deep groove from the body on the outer side and at each end. The groove is less apparent on the median aspect. The whole is covered with hairs of various lengths.

From an external view there is little to suggest any function for these peculiar organs. At times when the head is retracted it rests upon these bodies much as one's head rests against the curved supports at the back of a dentist's chair; but the patagia can hardly be there to support the head. A thinning of the cuticle over a small area near the lower, inner end affords some clue to the problem. Continuous with this thinning is a strand or ridge which runs downwards and inwards towards the base of the first thoracic legs (Fig. 15, Pl. IX.). From the inner surface of each patagium a very large number of muscle fibres arise, which come together into something like a tendon which passes along this ridge. It will probably be necessary to return again to this point in the description of the muscles, but at present we are inclined to regard the patagia as arrangements for increasing the surface of attachment of a powerful muscle which runs to the coxae of the first or prothoracic legs.

III. The Abdomen.

The abdomen of *Anopheles* consists of eight segments, each composed of a dorsal chitinous plate, the tergum, and a ventral chitinous plate, the sternum. Between the edge of the terga and sterna is a soft membrane, the pleuron (Fig. A). In the imago which has just appeared from the pupa the abdomen is very much longer than it is later. It stretches far beyond the wings, the ends of which only just reach the eighth tergum. In the older female imagines the abdomen is much contracted, and about a third, sometimes more, of the wing projects beyond the posterior limit of the body. In the male imago the wing-tips do not project beyond the last abdominal segment. The newly appeared imago also shows very clearly the pleura, which in fact project as a roll on each side of the abdomen, and in this state it is possible to see the minute abdominal stigmata, one on each side of the 2nd, 3rd, 4th, 5th, 6th and 7th segments, in each case a little in front of the centre of the segment. The number of abdominal stigmata is a

rather debatable point. Christophers says there is "one in each segment," and he draws in one figure seven and in another eight. The most careful search has only revealed six pairs to us and we do not believe there are more. They are however very difficult to see. When the imago is older the terga and sterna approach and overlap, and the pleuron and stigmata are hidden: the segments are also telescoped into one another for a considerable extent. In the imago which has just escaped from the pupa-skin the junction of the abdomen and the thorax is in a plane at right angles to the longitudinal axis of the mosquito, but as the thorax consolidates the pyramidal shape becomes emphasized and the plane of union is drawn forward and downwards. This drags the sterna forwards and produces what geologists would term a "fault" between the anterior terga and the anterior sterna. The first sternum is so pressed under the sloping metathorax that it lies almost entirely in front of the first tergum, and the limits of sterna and terga are not found in one plane until the fourth or fifth segment is reached; the sterna of the first three or four segments lie all somewhat in front of their corresponding terga.

From the surface of the terga and sterna numerous hairs project arranged in more or less transverse rows. These are more conspicuous on the dorsal than on the ventral surface, and most conspicuous in newly emerged imagines. The absence of scales on this part of the body is as Theobald (1901) points out a feature of generic importance. Projecting from the posterior end of the last segment in the male is a pair of stout basal lobes covered with hairs. In the imago as it appears when quitting the pupa these basal lobes seem to arise from the sternum, but in imagines of an older birth they seem to be borne by the terga. Each terminates in a long, yellow, chitinous claw—the clasper—tipped with black. The size and shape of these claws is of some systematic importance. The two claspers are usually folded over one another as a man's arms are crossed in the traditional attitude of resignation. This crossing is usually ventral, but it may in rare instances be dorsal, and this usually is the case in newly emerged imagines. On the inner side of the basal part, which is packed with muscles, there is a stout spur or spine which prevents the base being bent inwards beyond a certain point. During life the claspers are capable of a good deal of movement and are doubtless essential organs of copulation. In one male which lay a-dying the basal lobes were periodically thrown out till they lay almost at right angles to one another. The basal lobes then twitched four or five times per second and then the claspers were divaricated. It appeared as if the muscular effort was that of opening

not of closing the hooks. Miall and Hammond (1900) draw attention to the fact that such claspers as are here described do not occur in those insects where the female is provided with an ovipositor. In between the bases of these organs there is another set of very minute hooks and a very complex internal skeleton which supports part of the reproductive apparatus. The rectum is sometimes prolapsed for a short distance between the claspers. In the female the claspers are absent but their place is taken by two lateral flaps which probably play some part in oviposition.

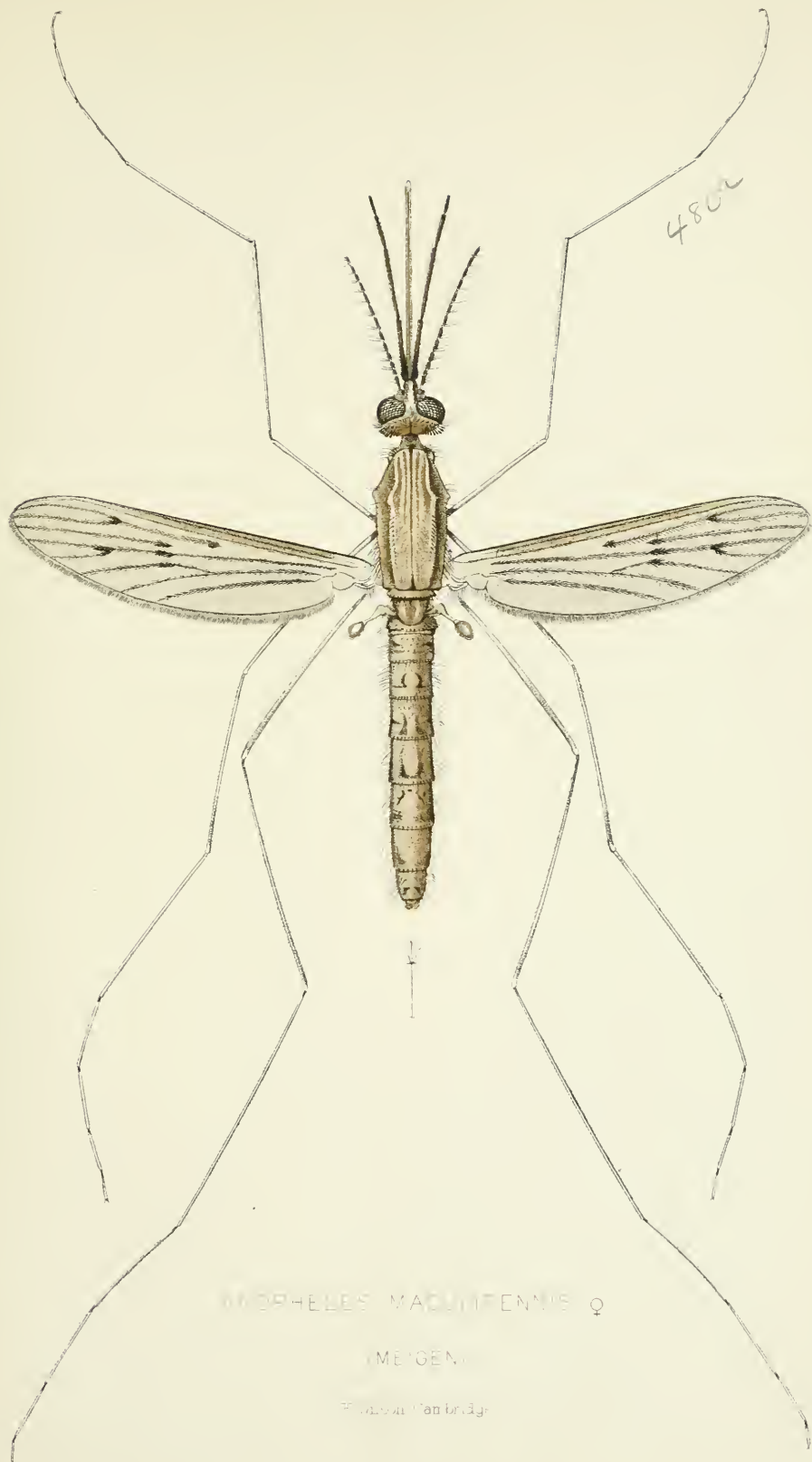
Hairs and Scales.

The body of *Anopheles maculipennis* is scattered over with hairs and scales. In some places, *e.g.* the legs and labium, the scales are so thickly placed that they conceal the structure which bears them, in other places, *e.g.* the head and wings, the scales are comparatively sparsely scattered and overlap but slightly or not at all.

The hairs are usually scattered, sometimes in rows. The longest are those of the male antennae, the stoutest those of the scutellum. Each hair arises from a thin, circular area in the chitin, which, when the hair is knocked off, remains conspicuous. The hairs are in all cases simple and show none of the complex beauty of the branched or palmate hairs of the larva.

The scales have their origin in a similar thin spot, but a much smaller and less conspicuous one. The scales have two or three varieties in their shape, and since Theobald has shown their great systematic importance it is worth saying a few words about them.

The scale of the wing is almost invariably lanceolate-shaped: a few however broaden out towards the free end, which, however, remains rounded. This latter shape is the common one for the scales of the legs, the palps and the labium. The scales of the head stand upright, well separated one from another and slightly arching forward so as to overhang the eyes. They have straight sides, and the end of the scale is either cut off straight or shows a reentrant angle which is intensified by a slight groove in the scale. All the scales are striated, and the thickenings which form the striae seem to project beyond the level of the end of these scales like a ragged fringe. In *A. maculipennis* we saw none of the minute scales which Theobald figures at the base of these cephalic scales, nor did we find any on the scutellum. In fact on the thorax proper and on the abdomen we saw no true scales, though hairs are abundant.



ANOPHELES MACULIPENNIS ♀

(MEIGEN)

From a fly net.

EXPLANATION OF PLATES.

Illustrating the Paper of G. H. F. Nuttall and A. E. Shipley on "The Structure and Biology of *Anopheles maculipennis*," Part III.

EXPLANATION OF THE ABBREVIATIONS USED ON PLATE IX.

<i>a.</i>	Antennae.	<i>mn.</i>	Mandible.
<i>al.</i>	Alula.	<i>m. p.</i>	Maxillary palps.
<i>ao.</i>	Anterior opening of the tubular passage through the head.	<i>ms.</i>	Mesosternum.
<i>ap.</i>	Apodeme of 1st maxilla.	<i>mt.</i>	Metasternum.
<i>b. a.</i>	1st segment of antenna.	<i>mus.</i>	Muscles.
<i>b. l.</i>	2nd segment of antenna containing auditory apparatus.	<i>mx.</i>	1st maxilla.
<i>cl.</i>	Clypeus.	<i>p.</i>	Patagium.
<i>co.</i>	Coxa.	<i>po.</i>	Posterior opening of the tubular passage through the head.
<i>c. s.</i>	Cephalic scales.	<i>pr.</i>	Proboscis.
<i>e.</i>	Epipharynx.	<i>Pr.</i>	Prosterna.
<i>em.</i>	Empodium.	<i>ps.</i>	Prosternum.
<i>e. p.</i>	Extensor plate on foot.	<i>r.</i>	Ridge connecting right and left mesosterna.
<i>f.</i>	Femur.	<i>s.</i>	Strut of tubular passage through the head.
<i>fl.</i>	Flange round pharynx from which the muscle to the hypopharynx arises.	<i>sal.</i>	Salivary duct.
<i>h.</i>	Hairs with collared sockets on foot of male.	<i>sc.</i>	Sclerite of neck.
<i>ho.</i>	Hooks on feet.	<i>sp.</i>	Triradiate sucking pharynx.
<i>hp.</i>	Hypopharynx.	<i>sq.</i>	Squama.
<i>l.</i>	Labrum.	<i>t.</i>	Trochanter.
<i>læ.</i>	Labrum + epipharynx.	<i>tr.</i>	Trachea.
<i>la.</i>	Labellae.	<i>tu.</i>	Tubular passages through the head.
<i>li.</i>	Labium.	<i>x.</i>	Space between clypeus and base of proboscis.

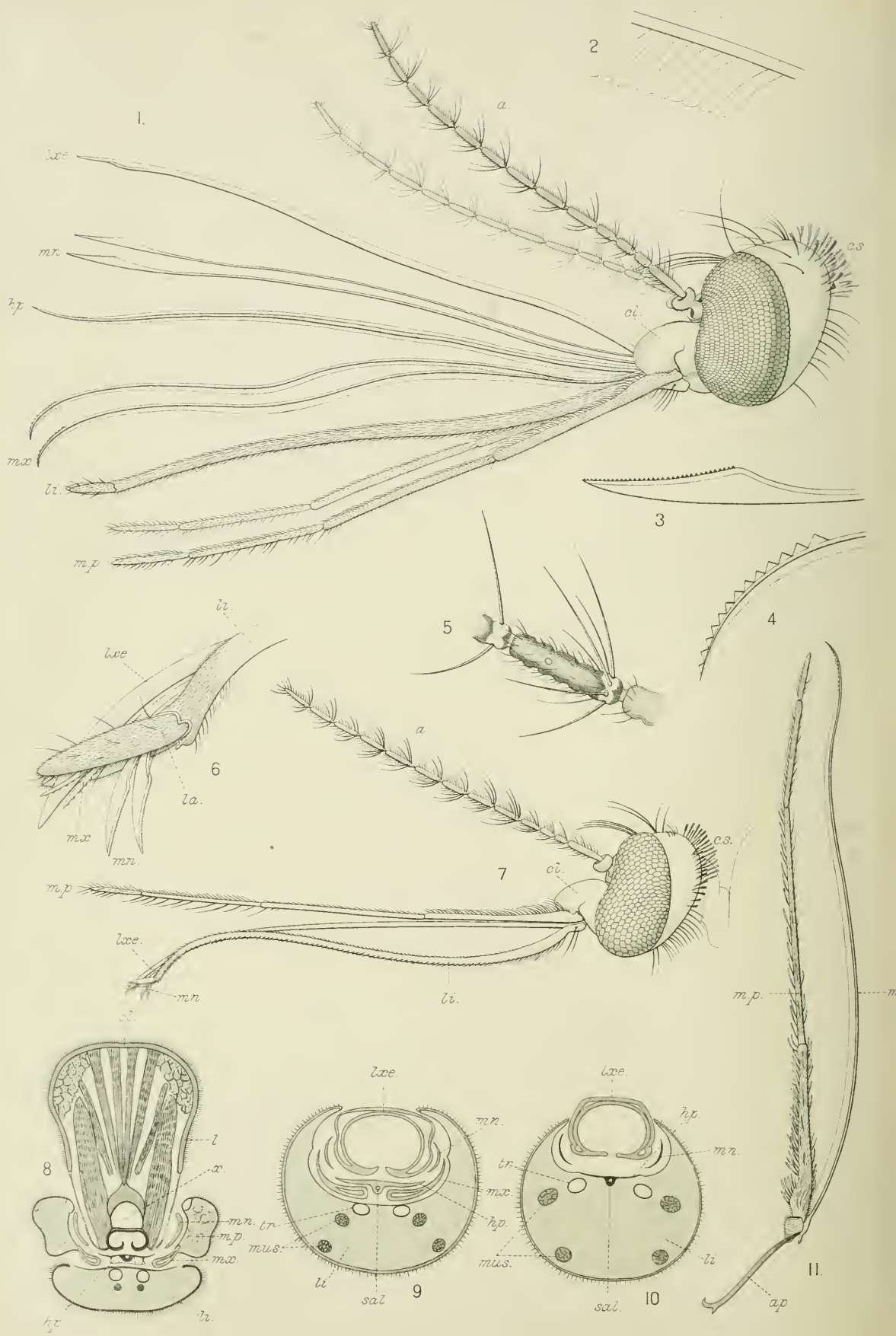
PLATE VIII.

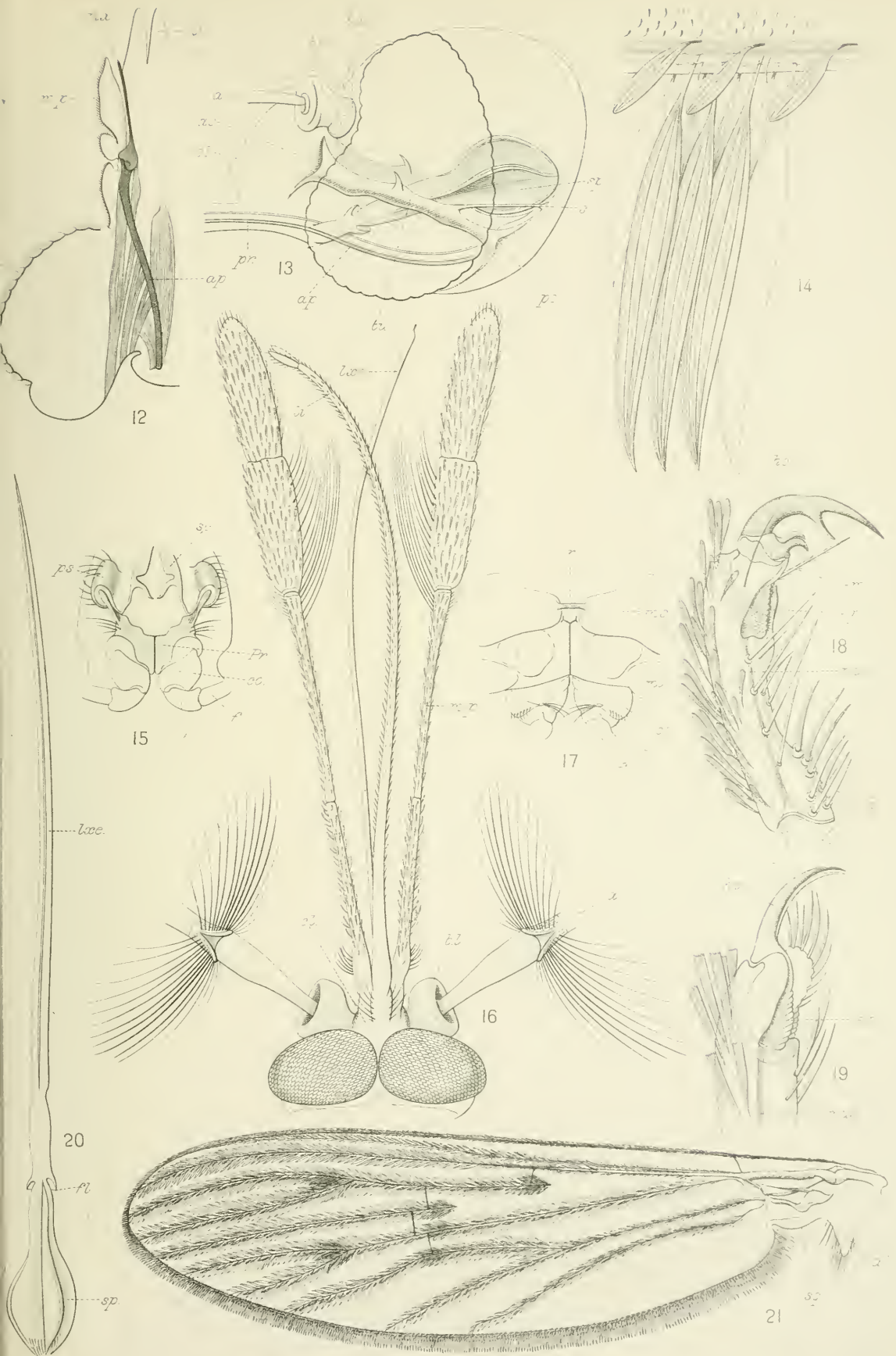
Anopheles maculipennis, dorsal view of female $\times 9$.

PLATE IX.

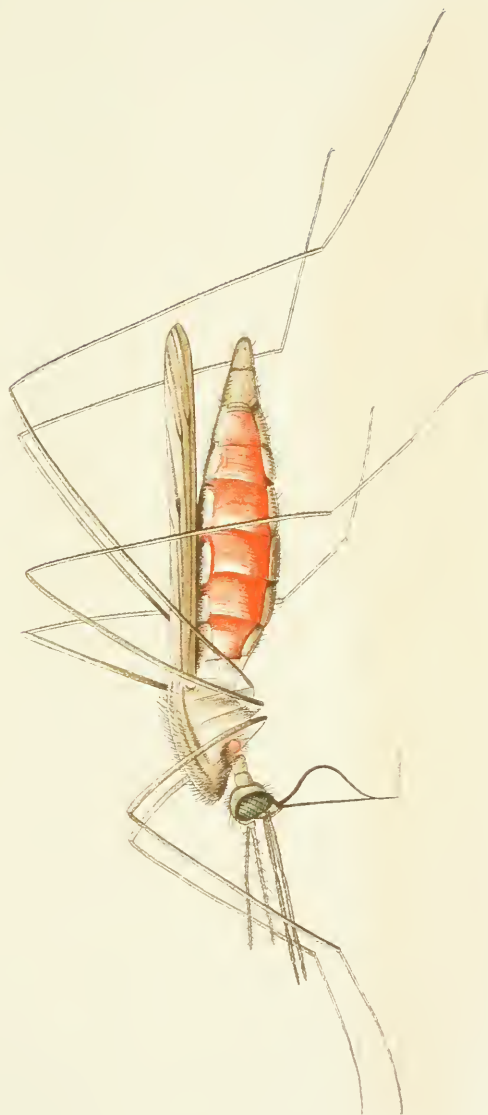
- Fig. 1. Side view of the head of a female *Anopheles maculipennis* \times about 40, with the various mouth parts separated but in the relative position in which they lie when enclosed in the groove of the labium. This figure shows the characteristic cephalic scales.
- Fig. 2. A small portion of the maxilla very highly magnified to show the blade with its characteristic areas.
- Fig. 3. The very finely toothed, sawing tip of the mandibles, very highly magnified.
- Fig. 4. The much more coarsely tooth tip of the maxillae, very highly magnified.
- Fig. 5. A segment of the antenna of a female *Anopheles maculipennis* showing the area free from pigment which bears the circlet of hairs.
- Fig. 6. A side view of the labellae and piercing organs of the proboscis of a female *Anopheles maculipennis* dissected out to show the tips of the mandibles, maxillae and labrum + epipharynx. The hypopharynx is not shown.
- Fig. 7. Side view of the head of a female *Anopheles maculipennis* \times about 26, showing the mechanism of biting. The labium is being curved and the labrum + epipharynx and the mandibles are appearing between the labellae at the tip of the labium.
- Fig. 8. Transverse section through the base of the proboscis and maxillary palps close to the anterior end of the clypeus and through the point of origin of the maxillary palps of a female *Anopheles maculipennis*. Muscles are shown running from the dorsal part of the clypeus to the ventral part and to the epipharynx. The labium has not yet become grooved. The space marked x is outside the body and represents the section of the chink between the lower anterior part of the clypeus and the origin of the labrum + epipharynx.
- Fig. 9. Transverse section through about the middle of the proboscis of a female *Anopheles maculipennis* showing the relative position of the parts when at rest. Two tracheae and two pairs of extensor and flexor muscles are seen in the labium.
- Fig. 10. Transverse section through about the middle of the proboscis of a male *Anopheles maculipennis*. The hypopharynx is fused with the labium and there are no mandibles.
- Fig. 11. Left first maxilla and its palp dissected out to show the connection of the two and the stout apodeme for the attachment of muscles, which traverses the head.
- Fig. 12. A horizontal section through the ventral portion of the head of *Anopheles maculipennis* showing the origin of the first maxilla and its palp and the strong apodeme. This traverses the head and has numerous muscles which arise from the skeleton of the head inserted along its course. It moves the maxilla.
- Fig. 13. A side view of the head of a female *Anopheles maculipennis*, which has been boiled in potash so that only the chitinous parts are left, to show the tubular passages and their openings, the apodemes of the maxillae and the triradiate sucking pharynx.
- Fig. 14. A portion of the hinder margin of the wing of *Anopheles maculipennis* highly magnified to show the arrangement of the fringing scales. The tissue of the wing is shown above. The large scales arise from the upper side of the edge, the medium sized scales which alternate with them from the under surface of the edge, and the

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ANOPHELES MACULIPENNIS ♀

COLLECTED BY

F. Wilson (Cambridge)

small scales which overhang the bases of the large scales from a ridge on the upper surface.

- Fig. 15. The under surface of the neck and prothorax of *Anopheles maculipennis* to show the patagia and the processes running down from them towards the coxae of the anterior pair of legs. The thin skin of the neck which permits the red of the blood to be seen when the insect is biting lies in front of the prosterna.
- Fig. 16. Ventral view of head of male *Anopheles maculipennis* showing the approximation of the eyes and the under surface of the clypeus, the mouth organs and the arrangement of the auditory hairs on the antennae.
- Fig. 17. A ventral view of the mesothorax and base of the metathoracic legs, showing the bridge clamping the mesosterna together. The second pair of legs have been removed.
- Fig. 18. A side view of the terminal tarsal segment of the first legs of a male *Anopheles maculipennis*, showing the hollow, the peculiar collared hairs, the 'extensor plate,' the retractor muscle and the complex hook.
- Fig. 19. A side of one of the legs of a female *Anopheles maculipennis* showing the double hooks, 'extensor plate' and muscle.
- Fig. 20. Labrum+epipharynx and pharynx and sucking pharynx. The groove of the labrum+epipharynx is shown here to pass into the tube of the mouth and pharynx.
- Fig. 21. View of wing of female *Anopheles maculipennis* showing the arrangement of the scales.

PLATE X.

Anopheles maculipennis, side view of a female $\times 7$, in the act of feeding on a human arm.

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OBSERVATIONS ON THE RECURRENCE OF DIPHTHERIA IN CAMBRIDGE IN THE SPRING OF 1901.

By LOUIS COBBETT, M.D., F.R.C.S.

(From the Pathological Laboratory of the University of Cambridge.)

THE outbreak of Diphtheria which occurred in Cambridge and Chesterton last October and November has already been the subject of a paper in this Journal, which dealt with the facts observed up to January 5th, 1901. The present communication deals with a return of the disease in the Spring of this year.

The force of the autumnal outbreak fell in the last two weeks of October and the first week of November, during which period 50 cases were notified and 5 deaths occurred. The prospect was disquieting, but the disease quickly subsided. In the course of the next week there were only 8 notifications; and from this time onwards until Jan. 5th but 8 cases occurred, 6 of these about the end of December.

During the six weeks which followed January 5th 5 cases of diphtheria were notified in Cambridge and Chesterton. In the week ending February 23rd there were four, but they were not confirmed by bacteriological investigation. After this a few cases continued to be notified each week, a maximum of eight being reached in the week ending April 6th.

Since April 15th up to the time of writing (Aug. 7th) there have been 7 notifications only, 3 unconfirmed and 4 confirmed by bacteriological investigation, the latter including a nurse in the diphtheria ward of Addenbrooke's Hospital, and one imported case.

The Spring outbreak may therefore be considered to have begun with the cases notified on February 19th and to have ended with that notified on April 15. The following table shows its progress.

TABLE I.

	Cases notified		B. Δ found			
Week ending Jan. 12	1					
19	1					
26	0					
Feb. 2	2					
9	0					
16	1					An imported case. Not including two country cases in Addenbrooke's Hospital
23	4	0	3	1 fatal		
March 2	1	1		1 fatal		Died after recovery from diphtheria of operation for removal of the tracheotomy tube
9	3	1	2			
16	3	3		1 fatal		
23	0					
30	4	3	1			
April 6	8	8		1 fatal		
13	3	2	1			Not including a country case in Adden. Hosp.
20	1		1			
27	0					
May 4	0					
11	0					
18	0					
25	1		1			
June 1	0					
8	0					
15	1	1				Nurse in the diphtheria ward Adden. Hosp.
22	1		1			
29	0					Not including a country case in Adden. Hosp. Probably an imported case
July 6	1	1		1 fatal		Not including a case notified as probably diphtheria and not confirmed by bacteriological examination
13	2	2				
20	0					
27	0					
Aug. 3	1		1			
	39	22	11			

The Spring outbreak produced in all 27 notified cases of diphtheria. All were bacteriologically investigated. By this means the diagnosis was confirmed in 18 cases (in 7 by microscopic examination of cultures only, and in 11 by the complete investigation of isolated cultures). In 8 it was not confirmed, though two or more cultures from each were examined¹.

Out of these 8 unconfirmed cases, one was probably a true case of

¹ From one patient who was dying when first brought under observation no swab was obtained.

diphtheria, for a brother and a sister at home who remained well, were found to have diphtheria bacilli in their throats. The remaining 7 were not clinically very typical, and may probably not have been true cases of diphtheria.

Among the 18 notified cases in which diphtheria bacilli were found were three deaths; but one of these was caused by an operation for removal of a tracheotomy tube long after recovery from diphtheria. If this one be excluded the case mortality was 11 per cent.; and this remains nearly the same if we include the 8 cases unconfirmed by bacteriological examination, of which one proved fatal.

During the Spring outbreak the same measures which had been used, as was thought with success, in the Autumn, were again put into practice.

(a) Antitoxin was supplied free for prophylactic use, in the case of those who had come into contact with the actual cases of diphtheria, or with those who, not being ill, were known to be harbouring the diphtheria bacillus. And in the case of the poorer classes prophylactic injections were offered and given by a medical man acting under the authority of the Medical Officer of Health.

(b) Swabs were supplied to medical practitioners, and bacteriological investigations of their poorer patients made at the public expense. Moreover, the medical practitioners were requested not to certify convalescents as free from infection until three consecutive negative examinations should have been obtained.

(c) Whenever diphtheria was known to have broken out in a school and the school had been accordingly closed, the children who had been attending, or such of them who belonged to the classes more particularly affected, were visited in their own homes by the Medical Officer of Health or his representative. And with the consent of the parents a bacteriological examination was made of the throats of them and of other children, if any were living in the same houses. The brothers and sisters of actual cases were sought out and examined as well as those who were in the habit of associating with the latter in work or play. When diphtheria bacilli had been found in any of those thus examined, the parents or guardians were told that the infected child was a source of danger and might communicate diphtheria to those with whom it came in contact, and they were advised to allow it to be isolated in a Home which was opened for the purpose. As nearly all the healthy persons found to be infected were children of school age or

less, it was not difficult to get consent to isolation¹. Seventeen healthy persons with diphtheria bacilli were discovered. Of these three were not isolated, because the Home was not then open, one because he was suffering from another contagious affection, and two only refused to go to the Home. The remaining eleven voluntarily submitted to isolation.

Besides the home examinations, on two occasions swabs were taken from children at schools where a notified case had occurred. In each instance, only those children attending the classes most implicated were examined, and no diphtheria bacilli having been discovered, these schools were not closed.

The work of visiting the school children in their own homes, of talking to parents, and getting them to consent to the examination of their children was very laborious, and required both tact and patience. And owing to the fact that the staff of helpers which was got together in October last was not available in the Spring, the work could not be carried out so thoroughly as was desirable. Nevertheless a large number of people were examined and the total number of swabs from all sources received since February has amounted to 466 (not including 153 from a country village). If we add those belonging to the Autumn outbreak, the total exceeds 1600, and 172 cultures have been isolated and tested on animals.

The following is an account of the distribution of the cases among the schools affected and the steps taken to prevent the disease spreading.

King Street School, Girls and Infants. Two children attending this school having been notified as suffering from diphtheria, 9 others belonging to the class affected were examined on February 20 with negative results. No diphtheria bacilli were found in the notified children nor in their brothers and sisters. The school was not then closed.

St Giles's Boys' School. A case of diphtheria having occurred in a boy attending this school, and another in the infant brother of a scholar, 32 boys were examined at the school on March 12, and cultivations made from their throats. In none was the diphtheria bacillus found. In no less than 17, however, were Hofmann-like forms present. Of these one, which more closely resembled the diphtheria bacillus, was isolated and tested for acid production and for virulence, with negative result. Those living in the same houses as the two patients, to the

¹ It was not thought expedient, as a rule, to examine the parents or bread-winners, on account of the impossibility of isolating them without provision being made for the support of those dependent on them.

number of nine, were also examined, with the result that the infant patient's brother who had been attending the school, and a brother and a sister of the other case, were found to be infected with the bacillus, though they remained well. These three children were supposed to be isolated in their own homes, the Isolation Home being as yet not available.

The school was not closed and no further cases occurred in it, nor in any persons connected with the patients or infected children.

King Street School, Girls and Infants. Several cases of diphtheria having occurred in this school since the examination mentioned above, the school was then closed and it was decided to make a bacteriological examination of the children in their own homes.

Owing to the difficulty of finding suitable assistants, this could not be completely or expeditiously carried out, and we had to be content with examining 63 out of the 160 children. As the cases were scattered widely throughout the school, it was impossible to select any particular classes as having been more exposed to infection than the rest. The result of this investigation was the discovery of three clinical cases of diphtheria not under medical treatment, and 10 healthy children with diphtheria bacilli in their throats. Cultures from 10 of these were isolated and tested, with the result that 6 were virulent to guinea-pigs, including those from the clinical cases. The other 4, which in their mode of growth on various media, in their reaction to Neisser's stain and in the production of acid when grown in glucose-broth, were identical with the diphtheria bacillus, nevertheless did not kill guinea-pigs in doses of 2.0 c.c. 48 hour old broth-cultures.

The cases and the infectious persons thus discovered were all isolated, either in the Hospital or the Home for persons infected with the diphtheria bacillus. No further case of diphtheria occurred at this school nor in any of those connected with the patients. It is, however, probable that the cessation of outbreak among the children of this school was not entirely due to the measures adopted. For since the investigation of about one-third of the school had revealed so many infected persons, it cannot be doubted that there must have been several infected persons also among the unexamined who were allowed to go free. That diphtheria stopped at this time is probably, therefore, to be attributed in a large measure to the closure of the school, and possibly also to the time of year¹. There were in all 13 cases of diph-

¹ The last case belonging to this school was notified on April 3. After this only five cases, two alone confirmed by bacteriological investigation, were notified in the

theria among the children attending this school, 3 unconfirmed by bacteriological examination, the rest all confirmed by complete investigation of isolated cultures.

A case of diphtheria occurred in a teacher in a High School for Girls. From this patient during the early stage of her illness a culture of non-virulent diphtheria bacilli was isolated.

Paradise Street School. Two notified cases occurred, but these were not confirmed by bacteriological diagnosis. Nine of the other children of this school were examined, but no diphtheria bacilli found.

The school was not closed; no further cases occurred.

St Barnabas. One unconfirmed case was notified.

British School. There were two cases, both confirmed, one fatal.

The Isolation Home.

The Home was opened primarily to accommodate those who, without being ill, were found to be carrying about the diphtheria bacillus. Eleven such were isolated. Lest any of them should at the time when they were examined have been passing through the incubation stage of diphtheria all received a prophylactic injection of antitoxin. In addition, owing to lack of other accommodation, two mild clinical cases of diphtheria and five convalescents from the Addenbrooke's Hospital were also admitted.

This association of healthy persons with cases of diphtheria was not undertaken without due deliberation. It was felt that the bacteriological examination could be firmly relied upon to exclude all except those who were harbouring the true diphtheria bacillus, and that such persons were not likely to be harmed by diphtheria bacilli received from others. Moreover, since Wasserman and others have shown that many human beings have in their blood a considerable amount of diphtheria antitoxin, it was thought that those who carry about the bacillus in their mouths without being ill, were probably protected in this way.

At the same time it was fully recognised that this argument does

town. The last confirmed case was notified on April 9th: the last case (unconfirmed) on April 15th. The weather, which had been cold and wintry, was very wet from the 10th to the 16th. After this it cleared up and became fine and dry, and for a few days very hot; so that we passed abruptly from winter to summer. The diphtheria, however, had ceased to spread a week before the change in the weather took place. The cessation of the outbreak therefore cannot be attributed to the change of weather, though it is not improbable that it was connected with some more subtle seasonal influence.

not necessarily apply to those persons who are found to be harbouring the non-virulent diphtheria bacillus. It may be questioned whether it is necessary to isolate these persons, and whether, if isolated among those who carry about the virulent diphtheria bacillus, they are not liable to catch diphtheria. In practice, however, the virulence of the bacillus is only determined after isolation has been carried out; and accordingly in our Home five of these persons lived for some time (see Table II.) in close contact with twelve others who were infected with diphtheria bacilli, known to be virulent in the case of eight. This action was followed by no bad results, no case of diphtheria or even of sore-throat occurring among the healthy persons in the Home.

From seven of the persons isolated in this Home, the bacilli were twice or oftener isolated and tested for virulence on the guinea-pig. The result was striking. Those admitted with a non-virulent diphtheria bacillus were never found to have acquired a virulent bacillus during their stay in the Home, nor was a non-virulent diphtheria bacillus ever found in a child in whom virulent bacilli had once been found. In the case of E. J. the bacilli were isolated and tested 10 times in the course of the 15 weeks she remained in the Home. During 5 of these weeks her little sister V. J. was with her constantly, and on three occasions the bacilli were isolated from her and proved fully virulent. Moreover from another girl, G. B., who remained in the Home almost as long as E. J., diphtheria bacilli were isolated and proved fully virulent no less than 6 times. The same evidence, though less strong, is afforded by the rest of the seven cases mentioned above. (See Table II.)

The conclusion seems to be either:

(1) that no transmission from one child to another occurred while they were in the Home;—and in this connection it must be remembered that antiseptics were in daily use by everyone, except for 24 hours before the taking of each swab; or else,

(2) that certain persons have the power of rendering a virulent diphtheria bacillus non-virulent when it gets upon the surface of the pharyngeal mucous membrane. And if we admit this, we ought also to admit that an attenuated bacillus recovers its virulence when it gets into the mouth of an ordinary individual.

This conclusion is supported by the fact that in two instances non-virulent diphtheria bacilli were found in a healthy elder sister of patients with actual diphtheria, and from whom virulent bacilli were isolated. It is tempting to conclude, either that the younger children suffered from diphtheria because they caught a non-virulent bacillus

TABLE II.

EXAMINATION OF NOTIFIED CASES, AND HEALTHY PERSONS FOUND TO BE INFECTED.

Δ=Diphtheria bacilli seen on microscopic examination.

Α=Diphtheria bacilli isolated and proved non-virulent.

Δ=Diphtheria bacilli isolated and proved virulent.

O=No diphtheria bacilli seen on microscopic examination.

+=Death of patient.

(A) *Notified Cases.*

Reference number and initials of patient	Date of notification	FEBRUARY					MARCH					APRIL					MAY													
		2	12	18	19	25	4	10	11	13	15	20	25	29	30	1	2	6	8	10	15	17	18	24	27	30	3	6	13	16
H. N.	2. II	Δ	O	-	O	-	O	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
E. M.	2. II	Δ	Δ	-	O	-	Δ	O	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
990 F. W.	1. III	-	-	-	-	-	Δ	-	-	-	Δ	O	O	O	-	-	O	-	-	-	-	-	-	-	-	-	-	-	-	-
939 M. A.	6. III	-	-	-	-	-	-	Δ	Δ	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
991 L. A.	10. III	-	-	-	-	-	-	Δ	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
937 M. A.	6. III	-	-	-	-	-	-	O	Δ	-	-	Δ	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
998 H. A.	26. III	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
938 R. A.	1. IV	-	-	-	-	-	-	O	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
981 Miss B.	12. III	-	-	-	-	-	-	-	Δ	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
1012 F. S.	29. III	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
1013 E. S.	1. IV	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
1015 J. M.	29. III	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
1023 E. T.	1. IV	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
1039 V. J.	1. IV	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
1038 J. H.	2. IV	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
1017 M. S.	3. IV	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
1066 M. M.	3. IV	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
1013 E. T.	4. IV	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
1101 Mrs G.	7. IV	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
1105 E. W.	9. IV	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
E. B.	19. II	-	-	+	O	O	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
898 C. B.	"	-	-	O	O	O	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
899 P. B.	"	-	-	O	O	O	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
901 T. B.	"	-	-	O	-	O	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
923 A. E.	6. III	-	-	-	-	-	O	O	O	-	-	O	O	O	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
1005 H. R.	27. III	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
994 G. Br.	6. IV	-	-	-	-	-	O	-	O	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
1047 S. W.	11. IV	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
1158 Mrs M.	15. IV	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

For further examinations in Isolation Home see below

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Most of the examinations of patients in Addenbrooke's Hospital were made by Mr T. Strangeways-Pigg, Pathologist to the Hospital.

(B) *Healthy persons found to be infected, but not removed to Isolation Home.*

- (a) Because the Home was not ready in time. (b) Refused to be removed.
 (c) Because suffering from contagious eczema.

	MARCH		APRIL						
	7	9	1	2-3	4-5	8	10	15	17
923 A. W. (a) brother to 990	Δ	Δ	O						
926 J. E. (a) brother and sister	-	Δ							
928 N. E. (a) to 923	-	Δ							
1025 M. R. (b)	-	-	-	Δ	-	-	O	-	O
1054 A. C. (b)	-	-	-	Δ					
1118 M. S. (c)	-	-	-	-	-	Δ			

(C) *Persons removed to the Isolation Home.*

- (a) Notified cases. (b) Convalescents. (c) Healthy persons found to be infected.

Reference number and initials of patient	APRIL										MAY					JUNE							JULY														
	3	6	8	10	15	17	22	24	26	27	30	3	6	13	16	18	23	25	28	1	5	7	10	16	24	26	28	1	3	5	9	10	13	19	22	23	
1066 M. M. (a)	-	Δ	-	-	-	-	-	O	O	Δ	-	Δ	-	O	O																						
1043 E. T. (a)	-	Δ	-	-	-	-	-	O	O	Δ	-	Δ	-	O	O																						
937 M. A. (b)	-	O	Δ	-	-	Δ	Δ	O	O	O																											
998 H. A. (b)	-	O	Δ	-	-	Δ	Δ	O	O	O																											
938 R. A. (b)	-	-	-	-	-	Δ	O	O	O																												
1039 V. J. (b)	-	-	-	-	-	-	O	Δ	O	O	Δ	Δ	Δ	O	Δ	-	O	Δ	O	O	Δ	(1)															
1034 E. J. (c)	Δ	-	Δ	-	Δ	-	Δ	Δ	O	Δ	Δ	Δ	Δ	O	-	O	Δ	O	O	Δ	Δ	Δ	Δ	Δ	Δ	Δ	Δ	Δ	Δ	Δ	Δ	Δ	Δ	Δ	O	O	
1099 G. B. (c)	-	-	Δ	Δ	-	Δ	Δ	O	Δ	Δ	Δ	Δ	Δ	O	-	O	Δ	O	O	Δ	Δ	Δ	Δ	Δ	Δ	Δ	Δ	Δ	Δ	Δ	Δ	Δ	Δ	Δ	O	O	
1026 F. S. (c) sister to 1012 & 1013	Δ	-	Δ	Δ	-	Δ	Δ	Δ	-	O	Δ	-	Δ	Δ	Δ	-	O	Δ	Δ	O	Δ	Δ	Δ	Δ	Δ	Δ	Δ	Δ	Δ	Δ	Δ	Δ	Δ	Δ	Δ	O	O
1028 L. W. (c)	Δ	-	Δ	-	O	O	-	Δ	-	O	Δ	-	Δ	Δ	Δ	O	Δ	O	Δ	O	Δ	Δ	Δ	Δ	Δ	Δ	Δ	Δ	Δ	Δ	Δ	Δ	Δ	Δ	Δ	O	O
1087 G. G. (c)	-	Δ	-	Δ	-	Δ	Δ	O	Δ	Δ	Δ	Δ	Δ	O	-	O	Δ	O	O	Δ	Δ	Δ	Δ	Δ	Δ	Δ	Δ	Δ	Δ	Δ	Δ	Δ	Δ	Δ	Δ	O	O
1082 E. D. (c)	-	Δ	-	Δ	-	Δ	Δ	O	Δ	Δ	Δ	Δ	Δ	O	-	O	Δ	O	O	Δ	Δ	Δ	Δ	Δ	Δ	Δ	Δ	Δ	Δ	Δ	Δ	Δ	Δ	Δ	Δ	O	O
1050 A. T. (c) sister to 1023	Δ	-	Δ	-	Δ	-	Δ	Δ	O	Δ	-	Δ	Δ	Δ	-	O	Δ	-	O	Δ	O	Δ	Δ	Δ	Δ	Δ	Δ	Δ	Δ	Δ	Δ	Δ	Δ	Δ	Δ	O	O
1090 M. H. (c)	-	Δ	-	Δ	-	O	O	O	O	O																											
1093 W. S. (c)	-	Δ	-	Δ	-	O	Δ	O	O	O																											
1117 M. H. (c)	-	Δ	-	Δ	-	Δ	Δ	O	O	O																											
1124 E. R. (c)	-	Δ	-	Δ	-	O	O	O	O	O																											

(1) This culture was more than once examined and no suspicious micro-organisms were seen: the child was consequently sent home. At a subsequent examination of the tube, however, one colony of diphtheria bacilli was found.

which regained its virulence when it entered their mouths, or that the elder girls caught a virulent bacillus from the children and caused it to lose its virulence. Either conclusion, however, would be at variance with the well-known stability of virulence, or want of virulence of the diphtheria bacillus¹.

On the other hand, if we conclude that those who are found to have the non-virulent diphtheria bacillus have acquired a bacillus already attenuated, we shall have to regard these persons not only as harmless to others, but as themselves liable to catch diphtheria when they come into contact with those who have the virulent diphtheria bacillus, for there would be no reason to regard them more than others as possessing a special resisting power. Neisser² indeed has recently found a high degree of antitoxic power in the serum of two girls who suffered from recurring sore throat and from whom the non-virulent diphtheria bacillus was isolated. But since diphtheria antitoxin is found in the serum of many ordinary people, it may in these cases have been quite unconnected with the fact that they harboured the non-virulent diphtheria bacillus. Lubowski (*loc. cit.*) attempted to produce antitoxin in rabbit with these same bacilli, but did not succeed.

While within the Home, those found to be harbouring the non-virulent diphtheria bacillus were not at any time separated from those who harboured the virulent bacillus.

Orders were, however, given that as soon as bacteriological examination had shown a child to be free from one or other of these bacilli, he should be removed to another part of the building and put with others in like case as himself, until three consecutive negative examinations had set him free, or the reappearance of the bacillus caused his return to the general part of the Home.

The experience gained during the Spring outbreak of diphtheria here, has tended to confirm the opinions arrived at during the Autumn outbreak.

I shall briefly refer to three of them.

(1) "Experience of the outbreak of diphtheria in Cambridge gave no reason for thinking that the pseudo-diphtheria bacillus is other than perfectly innocuous to man."

¹ Lubowski working in Ehrlich's laboratory (*Zeitschr. f. Hygiene*, Leipzig, Bd. xxxv., p. 87) found that he could not make non-virulent diphtheria bacilli virulent for guinea-pigs by repeatedly passing them through those animals.

² *Deutsche med. Wochenschrift*, 1900, Hf. 32.

During the Spring, as also in the Autumn examinations, Hofmann's bacillus was very frequently found. I do not know whether all bacteriologists would regard all the micro-organisms which I am accustomed to class under this head, as pseudo-diphtheria bacilli. Some, as they first appear on the original serum culture, are far more than others difficult to distinguish on morphological grounds from true diphtheria bacilli, and in not a few instances I have been in doubt until pure cultures had been isolated; but this I can affirm, that of all those isolated by me and tested on animals (17 since the beginning of March, and 69 last year), none form acid out of glucose nor produce any but a very transient local swelling when 2·0 c.c. of a well-grown 48 or 72 hour broth-culture is injected into the guinea-pig, and they do not give Neisser's reaction when grown for 22 hours on Löffler's blood-serum (ox). Moreover, however much they may have resembled the diphtheria bacillus at the start, they come in sub-cultures closely to resemble what I regard as the typical Hofmann form.

It has been thought better, for the sake of simplicity, to omit from Table II. all reference to the finding of Hofmann's bacillus, but it may be stated that it was frequently found, and that too, often at the time when the diphtheria bacilli were disappearing, and consequently not found without careful search. The two bacilli were often associated together and always with all their distinctive characters quite marked. From a child in whom the diphtheria bacillus had been found no less than 20 times, frequently isolated and tested for virulence, the diphtheria bacillus was found after prolonged search on the last occasion on which it appeared, and with it was the bacillus of Hofmann. Both micro-organisms were isolated. The Hofmann was typical in form, formed no acid, and was perfectly harmless to a guinea-pig which received 4·0 c.c. of a 48 hour broth-culture; while on the other hand 0·1 c.c. of a similar culture of the diphtheria bacillus killed a guinea-pig as usual within the 48 hours. There was therefore no evidence of the diphtheria bacillus becoming gradually changed into the pseudo-diphtheria bacillus just before its disappearance.

(2) It is, I believe, an error to conclude that diphtheria bacilli are distributed among the healthy members of a community free from diphtheria. These investigations have been made principally on children attending schools in which diphtheria had broken out, and on others who had been in more or less direct contact with actual cases. And so far as this was the case they afford but little evidence bearing on this point. But they include also the examination in November of

43 children attending a school in which there had been no case of diphtheria, and in March of 32 boys attending a school in which there was but one case, and of 9 boys attending another school in which there were two notified cases, neither of which was confirmed by bacteriological examination; and in none of these 84 children were diphtheria bacilli found. On the other hand, all the healthy persons who were found with the diphtheria bacilli in their throats had been in contact more or less directly with clinical cases. Thus of the 17 infected healthy persons discovered during the Spring, six were brothers and sisters of cases, two were girls employed at needlework in the same room as an infected person sister of a clinical case, nine were girls attending a school in which eleven cases of diphtheria had recently occurred. Thus all could be accounted for. And this remark is equally true of the healthy persons discovered to be infected with the bacillus during the Autumn. It may therefore be stated that *diphtheria bacilli were found in the healthy throats of those only who had come into more or less direct contact with actual cases of diphtheria*. On the other hand the bacillus of Hofmann was found quite as frequently among those who had never come into contact with cases of diphtheria as in those who had done so.

(3) Partially attenuated diphtheria bacilli have not been found.

As in the Autumn, so in the Spring, the cultures have either killed guinea-pigs within 48 hours, or three days at latest, the dose injected being 0.1 c.c. of a 48 hour broth-culture (or in some cases 0.5 c.c., this being the smallest dose injected), or 2.0 c.c. of a well-grown 48 or 72 hour broth-culture has produced nothing more than a trivial local lesion. The only exceptions to this rule have been two, and in each of these cases when the injection was repeated with a new culture, death took place within the usual time. I do not deny that diphtheria bacilli may become attenuated, but think it interesting to note that in a somewhat extended experience partially attenuated bacilli have never been found. Fifty-five diphtheria cultures have been separated and tested for virulence during the spring, making with the 24 isolated and tested during the autumn and winter, 79 in all.

It is also worthy of note that in no case, as far as is known, has a virulent diphtheria bacillus been replaced by a non-virulent diphtheria bacillus before its final disappearance. Reference to Table II. will show seven cases where the virulence of the bacilli present on from two to ten occasions in each case was tested and found constant.

The non-virulent diphtheria bacilli. Non-virulent bacilli were found during the Spring in one clinical case during the early stage of the

illness, and in 6 other persons who remained well. These bacilli were microscopically typical diphtheria bacilli and showed no points of distinction in their modes of growth on culture media. They gave Neisser's staining reaction. Only on the injection of animals did the difference show itself¹. The guinea-pig experiments were in the case of many of them repeated so as to leave no doubt as to the reality of the want of virulence. The animals injected with 2·0 c.c. of broth-culture suffered very little local swelling, and a small abscess about as big as a pea was the principal result². From these little abscesses the bacilli in pure culture were several times obtained and tested on guinea-pigs to see if they had gained in virulence. One of them was passed through four animals in succession. But in each case the injection of 2·0 c.c. of 48 hour broth-culture produced no more effect than at first.

It has already been stated that a harmless diphtheria bacillus was found during the early part of the illness in one case. A similar observation was made during the October outbreak, and in another case which occurred then, a non-virulent diphtheria bacillus was obtained from a patient examined for the first time during convalescence. Neisser³ points out that one cannot infer anything as to the virulence of a bacillus for man from observations on the guinea-pig, and refers to the culture of *Streptococcus* which Koch and Petruschky obtained from a woman who died of puerperal peritonitis, which as it was exalted in virulence for the rabbit, lost its virulence for man. On the other hand, I do not think it permissible to draw from experiences with the *Streptococcus*, inferences as to the diphtheria bacillus, which, unlike the coccus, forms *in vitro* a powerful poison which affects alike man and many animals. In Cambridge the non-virulent diphtheria bacillus has been found in 3 only of the 31 clinical cases which have been fully investigated since last October, while it has been found in no less than 8 out of the 18 persons who remained well, and from whom cultures of diphtheria bacilli were isolated. It would appear therefore that

¹ With two avirulent diphtheria bacilli of this kind Lubowski, *Zeitschr. f. Hygiene*, Bd. xxxv. p. 87, in Ehrlich's laboratory succeeded in immunising animals and producing a serum which agglutinated not only these bacilli but also 23 different races of quite typical diphtheria bacilli, but which had no action on pseudo-diphtheria bacilli.

² I have more than once seen similar abscesses form in guinea-pigs treated with large doses of virulent bacilli together with antitoxin. And also in an immunised horse treated with living bacilli. In the latter case the bacilli obtained from the abscess had retained their virulence.

³ Zur Differentialdiagnose des Diphtheriebacillus, *Zeitschr. f. Hygiene*, 1896, Bd. xxiv., p. 453.

the bacillus which is non-virulent for guinea-pigs is non-virulent also for man.

The non-virulent diphtheria bacillus occurred twice only among the seven diphtheria bacilli which were isolated from healthy persons in the Autumn, and the virulence of which was determined; while on the other hand it occurred 6 times out of 11 cultures of this kind obtained during the Spring. This may perhaps be due to some seasonal influence.

Should persons who without apparent illness are found to have the diphtheria bacillus in their throats be notified as cases of diphtheria?

Early in the course of the autumnal outbreak this question arose in Cambridge and was decided in the negative. The question may seem to some superfluous; the obvious answer being that diphtheria is a disease, and therefore a person cannot be held to have diphtheria, who remains well. But it has been urged on high authority, that these persons should be notified.

The question therefore seems worth discussing and that chiefly on the ground of expediency. It has been pointed out that without notification the Medical Officer of Health has no power to deal with these persons, but that armed with this instrument he can compel them to be isolated. In answer to this, it may be said that he could only compel the removal of those for whom it could be shown that isolation was impossible at home, and that too on the receipt of an order from a magistrate; that home isolation of a healthy person in a family which did not believe in its necessity could of course be nothing but a farce. Moreover compulsion in a few instances would raise a general opposition to isolation, and the taking of swabs, which could not be enforced, would be largely resisted.

On the other hand, when diphtheria is prevalent, the failure once and again to isolate a person in whom diphtheria bacilli have been found is not of great importance. It is clearly impossible to bacteriologically examine everybody who may have by some chance caught the bacillus. And since some such persons must inevitably remain at large, one more or less will not greatly signify¹.

Nevertheless it is worth while taking a considerable amount of

¹ This applies only to times when diphtheria is prevalent: at other times when none but sporadic cases occur it is possible, no doubt, to examine every 'contact,' and very desirable to isolate all infected persons.

trouble if we can only isolate a good proportion of these infectious persons, or at any rate keep them from school. In that case we should congratulate ourselves on our success and not grieve too much that some have escaped. The truth is we can do nothing unless the people back up our measures. Compulsion is fatal to success. If it were a case of dealing with our Public Schools and the class of people who send their sons to them, there would be little or no difficulty in carrying out bacteriological examination of contacts, and the parents would see to the isolation of their infected children, for they would at once recognise that the measures proposed were in their own interest. The poorer classes will take the same view if the matter is fairly explained to them. We must therefore in such matters act by persuasion rather than by force, and offer bacteriological examination as a privilege which it would be wise for them to accept, and let them refuse it if they will. That such a course is not barren of results is, I think, shown by the fact that we only once or twice met with a refusal to make an examination of children's throats, and that of the thirteen children whom we sought to isolate, permission was refused in the case of two only. One was the child of an ignorant woman who had strong opinions on the subject of compulsory vaccination. The other was a girl of 18 who would not be isolated because her people were just expecting visitors at Easter¹. Now had the Medical Officer tried compulsion, it is doubtful whether he would have succeeded in isolating these people. And the application of pressure would doubtless have stirred up to resistance others who were quietly complying with his recommendations.

¹ From both these persons who refused to be isolated, the bacillus in question proved to be a non-virulent diphtheria bacillus; and it is interesting to note that no case of diphtheria was known to arise from contact with either.

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